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INVESTIGATIONS of INDIANA LAKES AND STREAMS VOLUME VII

OCTOBER 1966

CONTENTS

1. Environmental factors affecting the local distribution and abundance of four species of stream-dwelling triclads
Clay M. Chandler 1
 2. The ecology of invertebrates in an intermittent stream
Hugh F. Clifford 57
 3. Coexistence in *Chaoborus* and its ecological significance
John B. Stahl 99
 4. The change from endogenous to exogenous sources of energy in bluegill sunfish larvae Dale W. Toetz 115
 5. Variations in productivity of Goose and hypereutrophic Sylvan lakes, Indiana Robert G. Wetzel 147
 6. Rate of digestion in the bluegill sunfish John Thomas Windell 185
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Environmental Factors Affecting the Local Distribution and Abundance of Four Species of Stream-dwelling Triclad¹

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ABSTRACT

The objective of this study was to define some of the factors in the lotic environment of four triclad species (Turbellaria), and to relate certain of these features to the local distribution and abundance of these planarians.

Using a time sample technique, 17 monthly collections of planarians were made in two streams about 5 mi west of Bloomington, Indiana. These observations showed that *Cura foremani* was abundant at the headwaters of Richland Creek but was replaced downstream by *Dugesia tigrina*. In Leonard Springs Branch, *Phagocata gracilis gracilis* occupied only the headwater and upstream areas and was replaced downstream largely by *Dugesia dorotocephala*. The latter species also occurred upstream but not at the headwaters.

Several physical and chemical parameters were investigated. Temperature, water level (discharge), substrate and changes in bottom character, calcium, and dissolved organic matter seemed directly related to the distribution and abundance of the triclads.

Continuous daily records and weekly maximum-minimum readings for one year indicated that the mean annual temperature fluctuation in both streams was less upstream (1.5-5.1°C) than downstream (5.6-8.8°C). Moreover, upstream areas had cooler summer means (15.4-18.7°) and warmer winter means (6.8-8.2°C) than those downstream (20.7-21.3°C; 3.0-4.9°C). That temperature influences distribution is indicated by the fact that all species except *D. tigrina* were comparatively more abundant in upstream areas of less temperature variability. The more exact nature of this relationship was revealed from laboratory observations that compared the acclimation capacity, reproductive capability, and general activity of each species over a range of 1-35°C. In general, the rather limited distribution of *P. g. gracilis* to cooler upstream areas seemed partly related to its apparently cold, stenothermal nature, and the similar distribution of *C. foremani* to its comparatively higher reproductive capability at lower temperatures. The scarcity of *D. tigrina* upstream as opposed to downstream seemed partly related to its comparative decrease in general and reproductive activity at lower temperatures. A relationship for *D. dorotocephala* was not readily apparent.

Biweekly observations of other parameters indicated that the relatively high calcium content of both streams (65-91 ppm) contributed to a chemically stable and generally productive environment. Dissolved organic matter was frequently less than 5 mg O₂/L absorbed from KMnO₄, which suggested no gross pollution. However, Richland Creek generally had higher values than Leonard Springs Branch, which could have excluded *D. dorotocephala* from Richland Creek, since this species is reputedly highly sensitive to pollution. There was a general inverse relationship between discharge and triclad abundance. High discharge in the spring suggests that

¹ Contribution No. 790, Department of Zoology, Indiana University. Based on a dissertation for the Ph.D. degree, Department of Zoology, Indiana University.

C. foremani was unable to maintain position in the otherwise slowly flowing headwaters of Richland Creek, which could have accounted for its absence from the continually swift current upstream in Leonard Springs Branch.

Flat, angular stones not easily moved by the current supported larger numbers of triclads than more rounded rocks that continually shifted. Only the upstream areas of Leonard Springs Branch had an abundance of stones covered with silt and CaCO_3 , which afforded a unique substrate for the unstalked egg capsules of *P. g. gracilis*.

The abundance of *P. g. gracilis* and *D. dorotocephala* in an apparent transition zone alternated reciprocally throughout the year, which suggested interspecific competition and migrational behavior in *P. g. gracilis*.

A field analysis of monthly changes in the size structure of the *P. g. gracilis* population suggested that breeding occurred mainly in autumn and winter and that hatching was predominantly in late spring and summer.

TABLE OF CONTENTS

	Page
Introduction	3
Description of the streams and stations	4
Leonard Springs Branch	4
Richland Creek	7
Description of the four species of triclads	10
<i>Cura foremani</i> (Girard, 1852)	11
<i>Dugesia dorotocephala</i> (Woodworth, 1897)	11
<i>Dugesia tigrina</i> (Girard, 1850)	12
<i>Phagocata gracilis gracilis</i> (Haldeman, 1840)	13
Methods	13
Physical methods	14
Chemical methods	15
Sampling techniques for triclads	15
Environmental observations	16
Distribution and relative abundance	16
Size structure of the triclad populations	19
Size structure for the entire sampling period	20
Monthly changes in size structure of the <i>P. g. gracilis</i> population at LS1	21
Relationship between <i>D. dorotocephala</i> and <i>P. g. gracilis</i> at LS4	22
Additional observations	24
Environmental observations on temperature, chemical features, and other physical features	26
Temperature	26
Observations based on continuous temperature records at Stations LS1, R2, and R3	26
Observations based on weekly readings of max-min thermometers at Stations LS4, LS2, R1X, and R4	31
Evaluation of temperature observations	32
Chemical features and other physical features	34
Water level and volume of flow	38
Calcium, total hardness, and magnesium	39
Chloride	40
Dissolved organic matter	40
Dissolved oxygen	40
Specific conductance	41
Turbidity	41
Substrate and changes in bottom character	42
Evaluation of observations on chemical features and other physical features	43
Laboratory observations	43
Temperature acclimation in the triclads	43
Acclimation to gradual changes of temperature	43

Acclimation capacity	44
Righting reaction time	44
Reproduction at different temperatures	47
Acclimation to sudden increases in temperature	48
Transfer from 13 to 25°C	49
Transfer from 13 to 29°C	49
Additional laboratory observations	49
Discussion	50
Temperature	50
Water level	52
Substrate and changes in bottom character	52
Chemical factors	52
Food relationships	54
Acknowledgments	54
References	54

INTRODUCTION

The objective of the present study was to define some of the factors in the environment of four species of stream-dwelling triclads and to relate certain of these features to the local distribution and abundance of these planarians. A considerable amount of information is available concerning the general ecology and distribution of freshwater triclad *Turbellaria* of the United States in Hyman's series of studies, summarized in her volume II of *The Invertebrates* (1951a), and in Kenk's descriptions of the triclad fauna of three states (1935, 1944, 1953). However, there is a dearth of specific ecological information on triclads of this country, especially the ecological factors that control local distribution.

A preliminary survey of two streams near Bloomington, Indiana, suggested that *Cura foremani* seemed to be abundant near the headwaters of Richland Creek, apparently being replaced downstream by *Dugesia tigrina*. In Leonard Springs Branch, *Phagocata gracilis gracilis* was abundant upstream but seemed to be replaced downstream by *Dugesia dorotocephala*.

Some of the many studies that demonstrate a relationship between temperature and the distribution of organisms in the lotic environment have been reviewed briefly by Macan (1961). The significance of temperature as a factor that limits the distribution of freshwater planarians has been repeatedly emphasized by a number of investigators (Beauchamp and Ulliyott, 1932; van Oye, 1941; Dahm, 1958; Reynoldson *et al.*, 1965). Published information on the thermal ecology of the triclad species of this study suggests that temperature is a potentially important factor (Mast, 1903; Behre, 1918; Hyman, 1925; Eddy and Gleim, 1932). Hence, particular attention was given to changes of water temperature on a daily, seasonal, and annual basis, and laboratory observations were made concerning the effects of temperature on the acclimation capacity, reproductive capability, and general activity of each species.

Current velocity and the nature and stability of the substrate are generally considered to be effective factors in determining the distributional patterns and abundance of stream invertebrates (Percival and Whitehead, 1929; Berg, 1948; Slack, 1955; Ambühl, 1959; Hynes, 1960). Current velocity *per se* was not studied here, but volume of flow (discharge) as reflected in changes of water level can be related to abundance

of triclads, and a similar relationship can be established for the type of substrate and the stability of the stream bottom.

Some aspects of water quality were investigated to ascertain if these features were affecting triclad distribution and abundance. Although the distribution of planarians is difficult to relate to physical and chemical parameters, some relationships have been found or suggested. Reynoldson (1958a, 1958b, 1958c) correlated the distribution of planarians in several lakes of England with certain chemical factors, notably calcium and dissolved organic matter, and van Oye (1941) found that the distribution of triclads was influenced by dissolved salts and pH. It has been suggested that *Dugesia dorotocephala* is limited to waters of high calcium content and cannot tolerate impurities in the water (Hyman, 1925). Jenkins (1964) discussed the possible effect of certain chemical factors on the reproductive processes of *D. dorotocephala*, with calcium being given considerable attention.

An analysis was made of the size structure of the triclad populations in order to complement the study of environmental features and to aid in the interpretation of field and laboratory observations on temperature. Moreover, such an analysis often aids in the elucidation of planarian life cycles, as shown in Reynoldson's studies on two triclad species in England (1960, 1961).

DESCRIPTION OF THE STREAMS AND STATIONS

Leonard Springs Branch

Leonard Springs Branch, a tributary of Clear Creek, is located about 6 mi southwest of Bloomington, Indiana, in the physiographic region known as the Mitchell Plain (Fig. 1). The principal sources of the stream are the closely adjacent Leonard Springs and Shirley Springs. These originate from a spring horizon that forms at the point of contact between the Salem limestone and the overlying St. Louis limestone (Marshall, 1935). Surface water from the drainage area enters sinkholes in the karst plain and flows through channels along the spring horizon, finally emerging from the base of a steep limestone hill. There is a rather extensive cave system associated with Leonard and Shirley Springs, and the cirque-like appearance of this headwater region suggests the term "steep-head valley" (Powell, 1961).

The gradient of Leonard Springs Branch is rather steep for the initial 0.2 mi of its course and is interrupted by several small waterfalls, ranging from approximately 1.5—6 ft in height. The flow in these upper reaches during periods of heavy runoff is torrential. The gradient becomes more gentle farther downstream as the stream winds through heavily wooded hillsides and then enters a swampy area. The latter represents a backwater behind the remnants of a former dam constructed in the 1920's for the purpose of forming a water-supply reservoir. The stream flows through a removed section of the old dam, cuts across wooded areas, cultivated fields, and pasture land, and finally empties into Clear Creek, some 2.6 mi from its headwaters at Leonard and Shirley Springs.

Several smaller streams and rivulets, charged with surface drainage during periods of heavy rainfall but dry for most of the year, join Leonard

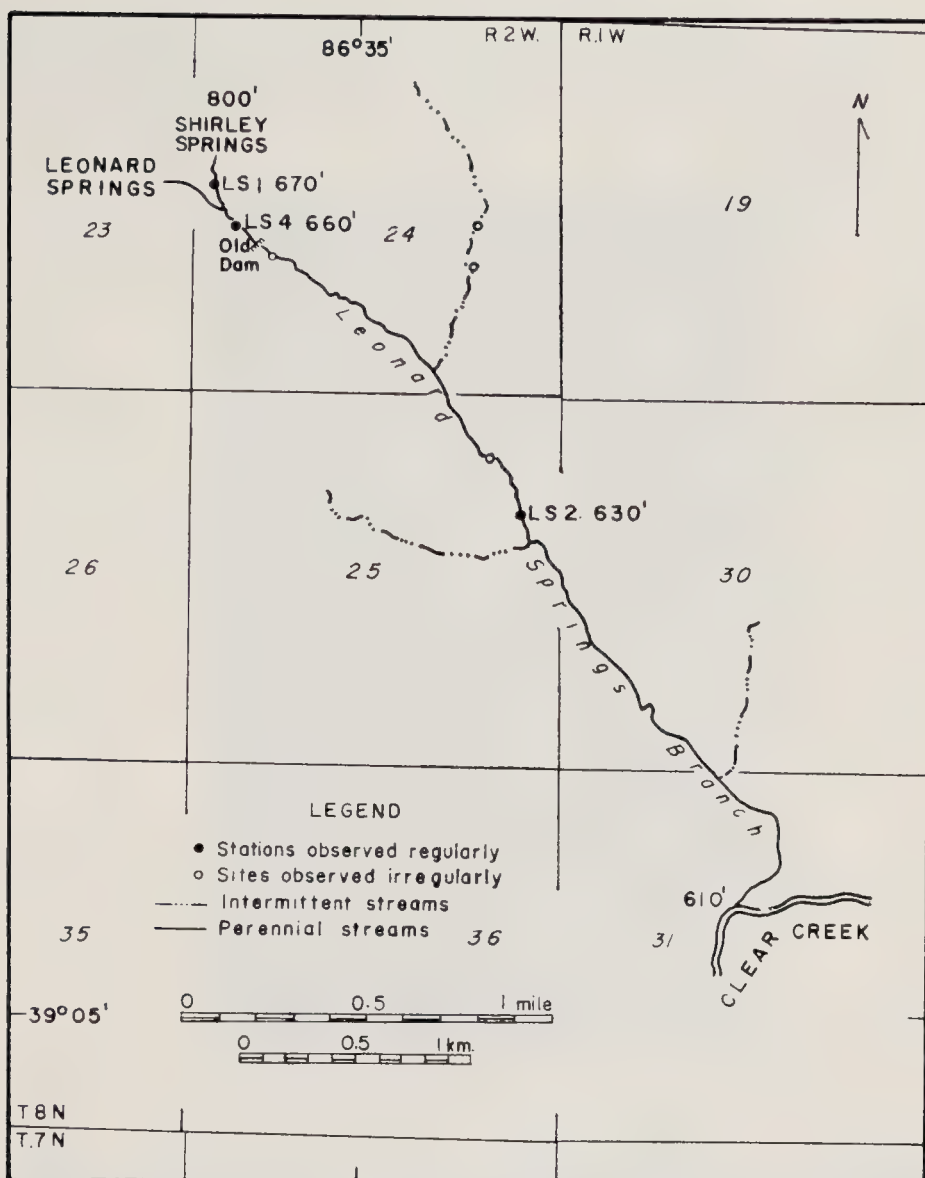


FIG. 1. Leonard Springs Branch, traced from U.S.G.S. Clear Creek Quad-range map, 7.5-min. series, 1956. Elevations other than bench marks approximately from contour intervals.

Springs Branch throughout its course. Only occasional observations were made in these small tributaries.

The selection of stations for intensive study was based on the numbers and species of triclads found during preliminary observations, and the contrasts in temperature, substrate, gradient, and accessibility. Station LS1 is located approximately 230 ft from Shirley Springs, in a turbulent portion of the stream about 40 ft downstream from a waterfall (Fig. 2). Station LS2, the one farthest downstream, is located near a bridge where the Rockport Road crosses the stream (Fig. 3). Later in the study, a third station, LS4, was established approximately 538 ft downstream from LS1; here the gradient is less steep and the stream less turbulent. All stations are characterized by alternating pools and riffles except LS1, where riffles predominate. LS2 has the greatest pooled area. The upper

portions of Leonard Springs Branch are choked with leaves and branches during the autumn months, and heavy growths of *Cladophora* cover the stones at LS1 during spring. Table 1 summarizes additional relevant information concerning stations.



FIG. 2. Station LS1 on Leonard Springs Branch.



FIG. 3. Station LS2 on Leonard Springs Branch.

TABLE 1. General characteristics of stations on Leonard Springs Branch (LS) and Richland Creek (R).

Station	Av. dimensions (ft) of section 20 ft long ¹		Banks ²	Shading	Bottom materials (most frequent type listed first) ³
	Width	Depth			
R1	—	—	Steep and 3 ft high at spring source	Exposed	Gravel, sand, and clay
R1X	—	—	Steep and 6-7 ft high on left bank at spring source; right bank sloping	Moderate; some trees and bushes on left bank	Rubble, boulders, and gravel
R2	8.1	0.6	Approximately the same as for R1X	Moderate; some small trees and bushes	Same as for R1X
R3	12.8	0.3	Steep and 2-3 ft high on left bank; right bank lower and of gentle slope	Slight; scattered small trees and bushes	Bedrock, gravel, and rubble
R4	15.1	0.6	Both left and right banks steep to sloping and 2-3 ft high	Slight; scattered trees and bushes	Gravel, rubble, sand, and clay
LS1	17.1	0.3	High (10-15 ft) and moderately steep on both left and right banks	Extensive; heavily wooded	Large flat rocks, rubble, and boulders
LS4	8.8	0.4	Steep and 3-4 ft high on left and right banks	Moderate; tall weeds, bushes and some trees	Gravel, sand, and clay
LS2	20.5	0.7	Moderately steep and 3-4 ft high on left bank; right bank lower and sloping	Moderate at first but later exposed after clearing	Gravel, sand, and rubble

¹ From field measurements of 28 March 1964

² Left and right banks are in the direction of stream flow

³ Bottom materials classified according to Roelofs (1964)

Richland Creek

Richland Creek, a tributary of the west fork of the White River, lies almost wholly in the Crawford Uplands near its point of contact with the Mitchell Plain (Fig. 4). In the upper reaches of Richland Creek about 4 mi west of Bloomington the stream has cut down through muddy sandstones and sandy shales of the Borden series (Knobstone), the streambed and basal part of the bluffs being composed of the St. Louis and Ste. Genevieve limestones (Malott, 1922).

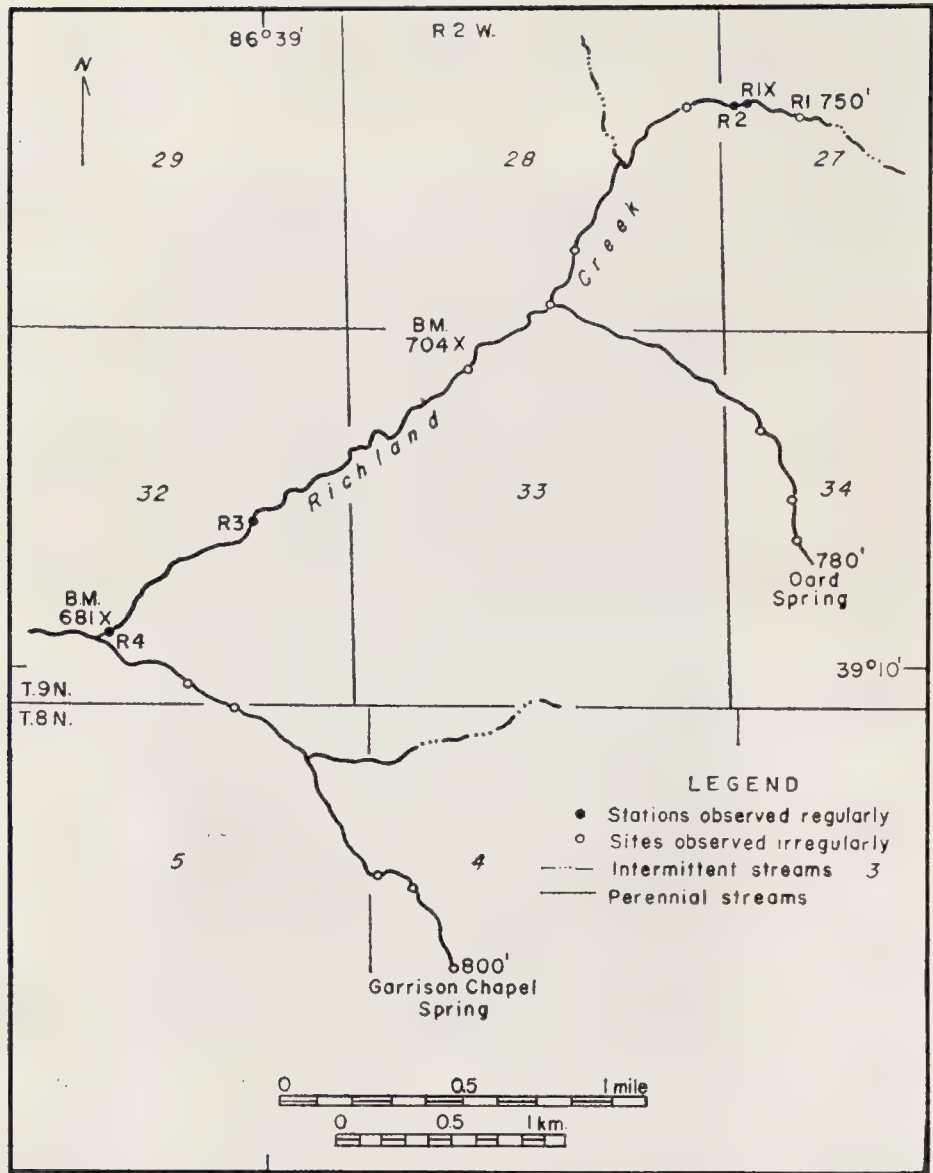


FIG. 4. Upper reaches of Richland Creek, traced from U.S.G.S. Whitehall Quadrangle map, 7.5-min. series, 1957. Elevations other than bench marks approximated from contour intervals.

Richland Creek has multiple sources, including several large springs, which are located in steephead valleys very similar to the sources of Leonard Springs Branch. Other sources are small seepage springs, which are the headwaters of some perennial tributaries of Richland Creek. These lesser sources received intensive study, whereas the larger springs were observed infrequently during this investigation. Only a steady trickle of water flows from the seepage springs, the turbulence that characterizes the larger springs being absent.

Stations for regular sampling and observation were located along approximately 2.7 mi of the upper reaches of Richland Creek; however, the entire course of the stream was traced during the summer of 1964, and observations were made at various points. The first 3 or 4 mi of Richland Creek flows almost entirely through pasture lands and cultivated



FIG. 5. Station R2 on Richland Creek.



FIG. 6. Station R3 on Richland Creek.

fields, receiving some waste material from septic tanks and cattle. The tributary from Oard Springs receives some waste material from the operation of a limestone quarry. The stream gradient of the upper reaches of Richland Creek is quite gentle, with no waterfalls such as appear in



FIG. 7. Station R4 on Richland Creek.

Leonard Springs Branch. Numerous intermittent tributaries join Richland Creek, but these were not regularly observed.

Station R1 located in a pasture at the source of a seepage spring had to be abandoned early in the study because of a small landslide that blocked the flow of the spring for several weeks. Station R1X was then established at the source of a similar spring in an adjacent pasture. Station R2 (Fig. 5) is approximately 50 ft downstream from Station R1X. Station R3 is near a bridge where Vernal Pike crosses Richland Creek (Fig. 6), about 1.9 mi downstream from R2. Station R4, approximately 0.5 mi from R3, was the farthest downstream station studied intensively; it is located at the bridge where State Road 48 crosses Richland Creek (Fig. 7). All stations are characterized by alternating pools and riffles, with a large pool existing at Station R4. Table 1 summarizes additional information concerning these stations.

DESCRIPTION OF THE FOUR SPECIES OF TRICLADS

The triclad species to be considered in this study are included in the Family Planariidae of the Suborder Paludicola.

The specific identification of planarians is made largely on the basis of reproductive structures, which require serial sections for their study. The flatworms were killed in a 1% solution of nitric acid, fixed with either a saturated mercuric chloride solution or Zenker's fixative, and stained with either iron hematoxylin and orange G or Mallory's triple connective-tissue stain. Transverse, sagittal, and frontal sections were made of several worms of each species. The diagnostic features of the reproductive systems, as confirmed by the sectioned material, are given in the accounts of the individual species.

Although this report is concerned primarily with the four species described below, a fifth species did occur at some stations. About midway through the study a small number of triclads that could not be

identified immediately began to appear in collections from LS1. Most individuals were quite small and immature, thus making positive identification difficult. Maintenance of these few specimens in the laboratory proved unsuccessful. Near the end of the study a few larger individuals, of apparently the same species as those collected earlier, appeared in collections. Serial sections of some individuals showed clearly an adenodactyl, a muscular gland organ situated posterior to the copulatory complex and diagnostic of the species of U. S. planarian, *Planaria dactyligera* Kenk, 1935.

Cura foremani (Girard, 1852)

C. foremani (Fig. 8) is a rather broad, plump flatworm that varies in color from uniform seal brown to black on the dorsal surface and lighter (grayish) on the ventral surface. Its length is up to 15 mm, and it varies in width from 2 to 3 mm. The head is of a low, triangular shape with a conspicuous unpigmented, diagonal stripe (auricular sense organ) at the base of each auricle. Two eyes are usual in each individual. The species is widely distributed in New England and Canada, west into Michigan and Indiana, and south into Tennessee and North Carolina (Hyman, 1959).

So far as known, reproduction in *C. foremani* is only sexual, although a capacity for regeneration is present. Sexual specimens can usually be collected at any time of the year. Stalked capsules are produced, and these are generally attached to rocks in the stream.

Some distinctive features of this species, as described and figured in Kenk (1935), are: (1) the absence of a copulatory bursa, (2) the connection of the bursa stalk with the intestine, and (3) the relatively small copulatory apparatus. Serial sections of my specimens reveal these features, and other observations agree with accounts and descriptions of the taxonomy and morphology of *C. foremani* found in Girard (1852), Curtis (1900), Hyman (1931, 1951b, 1959), and Kenk (1944).

Dugesia dorotocephala (Woodworth, 1897)

This is the largest freshwater planarian in North America (Hyman, 1959), some individuals from field collections being up to 25 mm in length and approximately 2 mm wide (Fig. 9). In well-fed laboratory cultures I have observed some worms as long as 40 mm. Specimens appear to the naked eye as uniformly dark brown to brownish black on the dorsal surface, the ventral surface being lighter in color. Under laboratory conditions of different temperatures the color is quite variable, being light tan and mottled in appearance at high temperatures. The head is definitely sagittate, and the auricles are very long and sharply pointed. There are two eyes situated slightly anterior to the level of the auricles.

Sexually mature individuals are seldom found in nature, and hence positive identification on the basis of reproductive structures is frequently impossible. None of the several hundred individuals collected during this study exhibited sexual reproduction, *i.e.*, no capsules were produced under any of the laboratory conditions. Moreover, no capsules were discovered in the field. No trace of reproductive organs was found in serial sections of the worms. Specimens maintained in laboratory cultures did undergo

prolific asexual reproduction by transverse fission, and many individuals from field collections exhibited blunt posterior ends, indicating that asexual reproduction was occurring in nature.

D. dorotocephala is apparently a polytypic species, which has resulted in some confusion concerning its taxonomic relationships. The identification as *D. dorotocephala* in this study is based on external features, behavioral characteristics, and distribution as described by Hyman (1925, 1929, 1931, 1959) and Kenk (1935, 1944).

D. dorotocephala is found most frequently in spring-fed streams and marshes from Pennsylvania and Virginia west to the Pacific coast (Hyman, 1959).

Dugesia tigrina (Girard, 1850)

This is the most common North American planarian, occurring in lakes, ponds, rivers, and streams from coast to coast in the United States (Hyman, 1959). The species reaches a maximum length of 18 mm, although most individuals observed in this study were from 10 to 11 mm. Although coloration and pattern of pigmentation are extremely variable, there seem to be two principal patterns, the striped form and the spotted form (Hyman, 1939). All individuals of *D. tigrina* observed in this study exhibited the spotted pattern, in which spots of different sizes and shades of brown form a background for irregular unpigmented blotches (Fig. 10). The ventral surface appears light gray to the naked eye. The head is triangular and has definite auricles, which are not nearly so long and

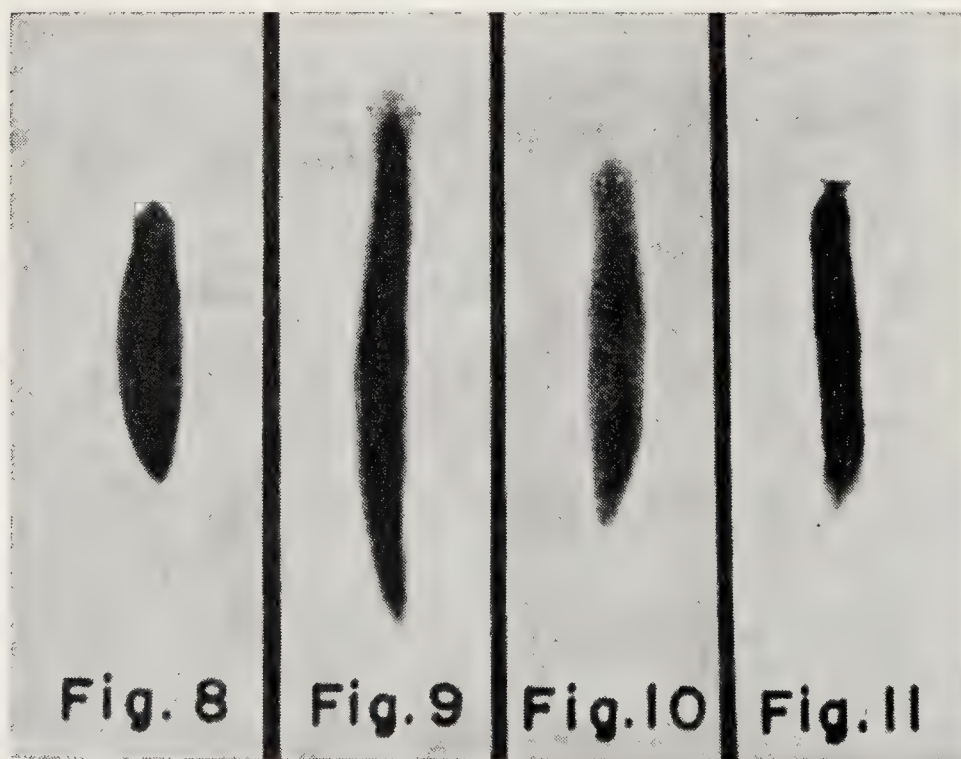


FIG. 8. *Cura foremani* from Richland Creek, x 2.9.

FIG. 9. *Dugesia dorotocephala* from Leonard Springs Branch, x2.7.

FIG. 10. *Dugesia tigrina* from Richland Creek, x 3.2.

FIG. 11. *Phagocata gracilis gracilis* from Leonard Springs Branch, x 2.5.

pointed as in *D. dorotocephala*. *D. tigrina* appears to be more sluggish than *D. dorotocephala* and glides more slowly than the latter when exposed to bright illumination. The body of *D. tigrina* is more compressed than *D. dorotocephala* from top to bottom, which may account for why it is more difficult to remove from the substrate in collecting.

The reproductive structures of *D. tigrina* and *D. dorotocephala* are very similar, the differences being mainly in the shape of the bursa stalk and the opening of the oviducts (Kenk, 1944). *D. tigrina* occurs in two distinct physiological races, an asexual race that propagates almost exclusively by transverse fission, and a sexual race that produces stalked capsules (Curtis, 1902; Hyman, 1939; Kenk, 1937, 1940, 1941). Capsules of *D. tigrina* were never found in the streams during this study, and no capsules were deposited in the laboratory. Since serial sections revealed no trace of reproductive structures, I assume that the asexual form is represented. Specific identification in this study was made by comparison with accounts in the above citations and in Kenk (1935) and Hyman (1951a, 1951b).

Phagocata gracilis gracilis (Haldeman, 1840)

This species is polypharyngeal and usually dark gray to almost black on the dorsal surface, although some individuals observed during this study were distinctly lighter in color, or exhibited various patterns of unpigmented areas (Fig. 11). The ventral surface is somewhat lighter than the dorsal surface. The truncate head is expanded at the anterior end to form two lateral auricles. Measurements of specimens from field collections indicate an average length of 10 to 12 mm with occasional specimens up to 15 mm.

Hyman (1959) recognizes three subspecies of *Phagocata gracilis*, viz., *P. g. gracilis* (Haldeman, 1840), *P. g. monopharyngea* Hyman, 1945, and *P. g. woodworthi* Hyman, 1937). The subspecies *gracilis* and *woodworthi* are indistinguishable externally. However, *gracilis* has a long, pointed penis papilla, and *woodworthi* has a short, truncate penis papilla (Hyman, 1937). Moreover, the two subspecies are largely allopatric, *gracilis* occurring from Pennsylvania and Virginia westward to Missouri and *woodworthi* from New England west to the Delaware River (Hyman, 1959). *P. g. monopharyngea* obviously has but a single pharynx, and by this feature could be distinguished from the other two subspecies. Specific identification in this study is based on a large penis bulb and a long penis papilla evident in serial sections, external features, and distribution, in comparison with accounts in the above citations and in Leidy (1847), Woodworth (1891), Peaslee (1910), Kenk (1935), and Hyman (1951a, 1951b).

METHODS

Each station was visited weekly, or occasionally biweekly, throughout the period of field study. Stations R1, R1X, R2, R3, and R4 were under observation from 12 October 1963 to 12 January 1965. Stations LS1 and LS2 were visited from 12 October 1963 to 3 April 1965. Station LS4 was observed regularly from 28 March 1964 to 3 April 1965 and was visited occasionally in May 1965.

Physical methods

Water temperature was measured continually at each of stations LS1, R2, and R3 with a recording thermometer (Dickson "Minicorder," The Dickson Co., Chicago, Ill.), which obtains a continuous 7-day record of temperature within a range of 20°-80°F. Weekly temperature extremes at stations R1X, R4, LS2, and LS4 were obtained by Taylor maximum-minimum thermometers anchored in the stream at each station. In spite of precautions to prevent breakage or vandalism of the max-min thermometers, some of this did occur, resulting in incomplete records for some weeks. The max-min thermometers and the temperature-sensitive probes of the recording thermometers were secured on the stream bottom and so positioned in the stream as to be largely protected from the direct rays of the sun. Both types of thermometers were checked throughout the study with a mercury thermometer mounted in a sling psychrometer. Records of air temperature and precipitation for the period of study were obtained from the Municipal Airport of Bloomington, an official U. S. weather station located approximately midway between Leonard Springs Branch and Richland Creek.

Water level was determined in two different ways depending on the location of the station. At stations LS2, R3, and R4 the distance from the stream bottom at midstream to a permanent mark on a bridge was measured and recorded on 6 November 1963. On return visits the distance from the permanent mark to the water surface was measured to the nearest 0.5 cm with a weighted, graduated copper chain. The difference between the original measurement and each subsequent measurement was recorded as the water level. At stations LS1 and R2, a staff gage, fashioned from a one-inch-diameter metal pipe and graduated in 0.5-cm intervals, was driven into the stream bottom at midstream until it reached bedrock. On 6 November 1963 the water depth at each staff gage was measured and recorded. The staff gage was then permanently marked slightly above the water surface, and appropriate values were assigned to graduations above the permanent mark. On subsequent visits values above the permanent mark were read and added to the original depth value; values below the permanent mark were determined with a mm rule and subtracted from the original depth value. The sum or difference was recorded as water level. In both ways of determination water level approximates water depth except as the elevation of the stream bed has changed between visits. It was not considered necessary to measure water levels at stations R1X and LS4 because of their proximity to stations R2 and LS1, respectively.

Volume of flow was measured according to the Embody method (Welch, 1948) at those times when it would best reflect comparison between normal and excessive flow.

Turbidity and specific conductance were measured in the laboratory. Turbidity (ppm silicon dioxide) was determined using a Hellige Turbiditymeter. Resistance (ohms) was measured with a conductivity bridge (model RC 16, Industrial Instruments, Inc.). Specific conductance (micro-mhos at 18°C) was calculated from the resistance by the procedure in Smith (1962).

Determinations of pH were made in the field using a Hellige Pocket Comparator (model no. 605-A) with "Bromthymol Blue-D" indicator solution.

Chemical methods

Water samples were obtained mostly at biweekly intervals at each station by completely immersing a 1-liter polyethylene bottle in the stream until it was completely filled, the bottle having been first rinsed twice with stream water. Great care was taken not to disturb the stream bottom during sampling. Water samples were taken from all stations in a single day and transported to the laboratory (being kept in an ice chest during warm weather), where they were placed in a 5°C temperature room in the dark to reduce biological activity to a minimum. The samples were usually analyzed within one week after collecting, and in no event later than two weeks.

Calcium, magnesium, and "total hardness" (calcium and magnesium together) were determined according to the methods in Mackereth (1963), with some modification because of the high calcium content of the water. Five ml of the water sample, diluted to approximately 25 ml with mineral-free water were used for each analysis of calcium and total hardness instead of 25 ml as recommended by Mackereth. Dilution of the sample resulted in a sharper end point than otherwise possible.

Dissolved organic matter was determined from the amount of oxygen absorbed from potassium permanganate as outlined in Mackereth (1963). Biweekly water samples were not diluted, and the sample volume for each analysis was that recommended by Mackereth (1963) for unpolluted waters. This method does not yield a strictly quantitative measure of the organic carbon present, but it does give useful results for comparative purposes. Fjerdingsstad (1950) showed that the potassium permanganate method for dissolved organic matter compared favorably with the standard method for determining biochemical oxygen demand.

Chloride content of the water was determined according to the Mohr method (APHA, 1960). Dissolved oxygen was determined in the field twice during the study (July and August 1964) according to the unmodified Winkler method.

Sampling technique for triclads

A time sample method similar to that used by Reynoldson (1958a) was employed for collecting triclads at each station in order that information concerning relative abundance and the population structure of triclads on a yearly basis might be obtained. Stones of rubble and gravel size were closely examined for the presence of triclads at each station for a minimum of 10 minutes. Sampling was concluded at a particular station if 100 or more triclads were collected at the end of 10 minutes. If 100 triclads were not obtained after 10 minutes, the search was continued until 100 were collected or one hour of collecting time had elapsed, whichever came first. Triclads were assumed to be absent in a relative sense if none were collected after one hour. For each collection a record was kept of the number of stones examined and the time required. Monthly

collections were made at all stations on the same day when feasible. After station R1 was abandoned, an initial collection was made at R1X in order to establish the presence of *C. foremani*. Thereafter, no regular collections were made at R1X because of its proximity to station R2. About midway in the study it was felt that too many triclads were being removed from some stations, and hence the sampling time and minimum numbers of triclads required for each collection were halved, *i.e.*, 5 minutes minimum time for 50 triclads and 30 minutes maximum time and/or a minimum of 50 triclads.

Each collection of triclads was brought to the laboratory, and shortly thereafter an examination was made to determine the species present, the number of individuals of each species, and the length of each individual. Worms were allowed to glide about in a large petri dish of water which was placed over graph paper ruled in 1-mm squares, the entire assembly being illuminated with a 60-watt incandescent lamp. Under these conditions a flatworm was usually extended fully, and its length could be measured to the nearest millimeter. Each collection was then placed in a white, enameled pan of charcoal-filtered tapwater and maintained at 13°C for further observation. Triclads were fed fresh beef liver usually once each week, and the water was changed at the completion of feeding.

ENVIRONMENTAL OBSERVATIONS

Distribution and relative abundance

The distribution and relative abundance of planarians at regular collecting stations on Leonard Springs Branch and Richland Creek (Fig. 12) is based on the average number of triclads collected per hour (relative abundance), being calculated for each species at each station as follows:

$$\frac{\text{total number of triclads collected}}{\text{total number of hours spent collecting}} = \frac{\text{number of triclads}}{\text{collected per hour.}}$$

Values used in the above calculation and the percentage of each species collected at each station are shown in Table 2. Species collected at a rate of less than 4 per hour at a particular station are not shown in Figure 12 but are included in Table 2.

Triclads are more abundant in Leonard Springs Branch than in Richland Creek, being especially abundant upstream in the former stream. *P. g. gracilis* occurs at LS1 almost exclusively and is also abundant under the stones at Shirley and Leonard Springs; it extends downstream as far as LS4, but was never collected beyond this point in the main course of Leonard Springs Branch. The total number of triclads collected per hour was greater at LS4 than at any other station in either stream, this station apparently representing a transition zone for *D. dorotocephala* and *P. g. gracilis*. The relationship between the two species in this zone will be described later.

D. dorotocephala occurs sporadically upstream from LS4 but apparently never occurs at LS1 or in the headwaters. A large population of *D. dorotocephala* appears immediately below the old dam, and the species extends from this point throughout the stream, being the most

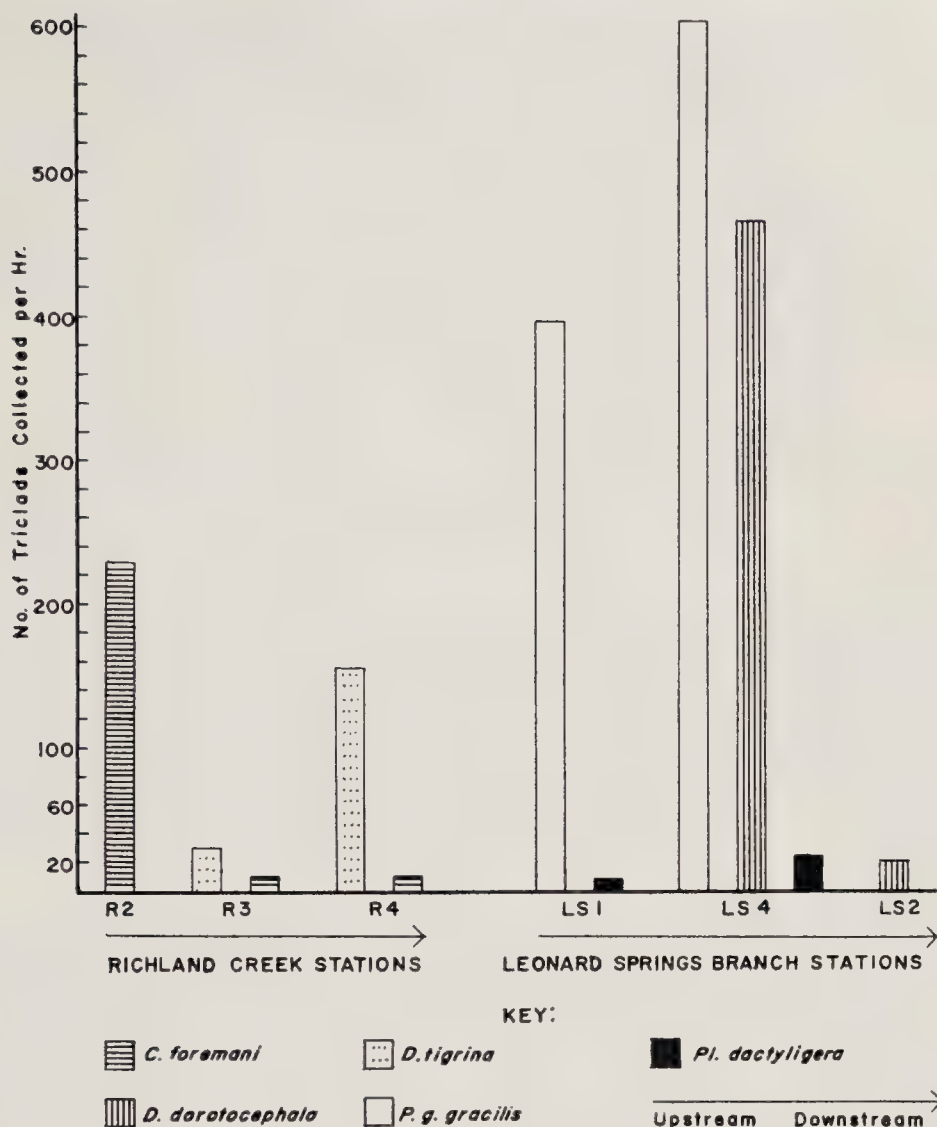


FIG. 12. Distribution and relative abundance (number of animals collected per hour) of five species of triclads at stations on Leonard Springs Branch and Richland Creek.

abundant triclad at LS2. Planarians are less abundant but more diversified as to species at LS2 than at any other station in either stream. This heterogeneity of species probably results from passive migration of triclads from tributaries and other areas of the main stream.

Only *C. foremani* and *D. tigrina* were collected in the main course of Richland Creek. Apparently, *C. foremani* exclusively occupies the upper reaches of Richland Creek (R1, R1X, R2) but decreases in abundance downstream to R3 and R4. Three monthly collections at R1 contained only *C. foremani*, and observations at R1X indicate that *C. foremani* is the only species at this station. *D. tigrina* is well established at R4, decreases in abundance at R3, and is found infrequently between R3 and R2.

Occasional observations in the tributaries of Richland Creek revealed some interesting distributional features. Oard Spring (Fig. 4) is situated in a steephead valley similar to that of Shirley Springs but on a smaller scale. A small stream originates at the spring, flows for about 75 yd, and

TABLE 2. Total number of triclads of each species collected (N), percent of each species at each station (%), and total number of hours spent collecting (Hrs) at stations on Leonard Springs Branch (LS) and Richland Creek (R).

Station	<i>C. foremani</i>		<i>D. dorotocephala</i>		<i>D. tigrina</i>		<i>P. g. gracilis</i>		<i>Pl. dactyligera</i>		Hrs
	N	%	N	%	N	%	N	%	N	%	
R2	954	100	—	—	—	—	—	—	—	—	4.1
R3	80	25	—	—	234	75	—	—	—	—	7.8
R4	59	7	—	—	873	93	—	—	—	—	5.6
LS1	—	—	—	—	—	—	1472	98	32	2	3.7
LS4	—	—	516	43	—	—	668	55	27	2	1.1
LS2	5	2	205	84	26	11	—	—	8	3	9.5

suddenly disappears through fissures in the limestone stream bottom. *P. g. gracilis* occurs under stones in the spring and stream. About 50 yd below the point of stream disappearance a small seepage spring restores flow in the Oard Spring tributary. *C. foremani* occurs in the small seepage spring, *D. tigrina* is found farther downstream, but *P. g. gracilis* is apparently absent. The spring at Garrison Chapel (Fig. 4) also harbors *C. foremani*.

The relative density of triclads at each station is obtained from the number of worms per stone (Table 3). For each station:

$$\frac{\text{total number of triclads collected}}{\text{total number of stones examined}} = \frac{\text{concentration of}}{\text{triclads per stone.}}$$

Relative density is greatest at the upstream stations of both streams but almost 2 to 3 times greater at LS1 and LS4 than at R2. The relative density of triclads at LS2 and R3 is very low but is considerably higher at R4, where *D. tigrina* occurs abundantly.

TABLE 3. Concentration of triclads per stone (relative density), total number of stones examined, and total number of triclads collected at stations on Leonard Springs Branch (LS) and Richland Creek (R).

Station	Concentration of triclads per stone	Total no. stones examined	Total no. triclads collected
R2	1.2	817	954
R3	0.2	1595	314
R4	0.9	1038	932
LS1	2.1	733	1504
LS4	3.8	316	1211
LS2	0.1	2376	244

The abundance of triclads at each station can be compared further by use of an abundance index, which was developed according to the arbitrary criteria in Table 4. The monthly abundance index and the average abundance index for each station again shows that LS1 and LS4 are most abundant in triclads (Table 5). Moreover, both stations supported large numbers of planarians throughout the collecting period. R2 and R4 have relatively high average abundance indices which are similar. However, monthly indices vary considerably at both stations. A relatively large number of planarians was initially found at R3, but they subsequently became scarce until Nov. and Dec. 1964. LS2 is characterized by a low abundance index throughout the collecting period.

The decrease in abundance at some stations may result partly from the considerable removal of triclads prior to modification of the sampling procedure. However, the effect of changes in water level accounts for some of the decrease, as will be discussed later.

Summarizing, there seems to be a definite pattern in the local distribution of these planarians. *P. g. gracilis* is abundant only in the upstream areas of Leonard Springs Branch, especially at the headwaters, and a similar situation obtains for *C. foremani* in Richland Creek. *D. dorotocephala* is more abundant upstream than downstream in Leonard Springs Branch, but never occurs at the headwaters. Although *D. tigrina* is found in downstream areas of both streams, it is most abundant in these areas of Richland Creek and is seldom found upstream.

Size structure of the triclad populations

Size structure of the populations is based on absolute numbers of planarians collected and is presented in two ways: (1) size structure of the population of each species for the entire collecting period at stations where more than 100 planarians were collected (Fig. 13), and (2) monthly changes in the size structure of the *P. g. gracilis* population at LS1 for one year (Fig. 14). In both instances the length of each uninjured planarian was measured as described under "Methods," and the number of triclads in each size group was divided by the total number of triclads collected to yield the percentage of each size group in the population.

The accurate assignment of age to each size group is not possible, but length can be correlated with degree of sexual maturity for *C. foremani* and *P. g. gracilis*. Squash preparations of frequently killed worms were made and microscopically examined for reproductive structures. Worms having a penis and associated reproductive structures were judged to be sexually mature. Examination of several worms in each size group and laboratory observations on breeding revealed that *P. g. gracilis* usually attains sexual maturity when 7 to 8 mm long, and *C. foremani* is generally sexually mature at 7 to 9 mm. On this basis the following relationships of length to degree of sexual maturity were arbitrarily assigned:

	<i>C. foremani</i>	<i>P. g. gracilis</i>
adult	> 9 mm	> 8 mm
young	7-9 mm	7-8 mm
immature	< 7 mm	< 7 mm.

TABLE 4. Criteria used for development of the monthly abundance index.

Abundance index	Number of triclads collected on basis of time sample technique	
5 (very abundant)	100 in 10 min.	or 50 in 5 min.
4	100 in 10-30 min.	or 50 in 5-15 min.
3	100 in 30-60 min.	or 50 in 15-30 min.
2	50-100 in 60 min.	or 25-50 in 30 min.
1 (scarce)	<50 in 60 min.	or <25 in 30 min.

Size structure for the entire collecting period. The size structures of the *P. g. gracilis* populations at LS1 and LS4 are distinctly different (Fig. 13). The histogram for LS1 shows a rather even distribution of adults (39%), young (32%), and immature (29%), whereas the histogram for LS4 is an inverted pyramid with 72% adults, 14% young, and 14% immatures. Apparently, breeding is more successful at LS1, since the percentage of young and immatures is more than twice that of LS4. The shape of the histogram of *C. foremani* at R2 is similar to that of *P. g. gracilis* at LS1, suggesting that this population is breeding successfully.

Length cannot be correlated with degree of sexual maturity for *D. dorotocephala* and *D. tigrina*, since both are asexual, and fission can occur at almost any length. In the absence of definite criteria for relating length to age, population structure is described in terms of larger or smaller planarians relative to average length of well-fed laboratory specimens. The greater percentage of smaller *D. dorotocephala* at LS2 compared to LS4 suggests that food for triclads may be less plentiful at LS2, since well-fed laboratory specimens average about 16 mm in length. The greater percentage of smaller *D. tigrina* at R3 compared to R4 suggests a similar interpretation, since the average length of well-fed laboratory specimens is about 10 mm.

TABLE 5. Monthly abundance indices and average abundance index for stations on Leonard Springs Branch (LS) and Richland Creek (R).

Stations	1963			1964												1965		Av. abundance index
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Feb	Mar	
R2	5	5	5	4	4	2	—	—	2	3	3	4	4	3	3	—	—	3.6
R3	4	1	—	1	1	1	—	—	1	1	1	1	1	3	2	—	—	1.5
R4	5	5	—	2	4	1	—	1	4	4	5	4	2	4	3	—	—	3.4
LS1	5	5	5	5	5	4	4	4	4	4	4	4	4	4	4	4	4	4.3
LS4	—	—	—	—	—	—	4	5	5	5	5	5	5	5	5	5	5	4.9
LS2	1	2	—	1	1	1	—	1	1	1	1	2	1	1	2	—	—	1.2

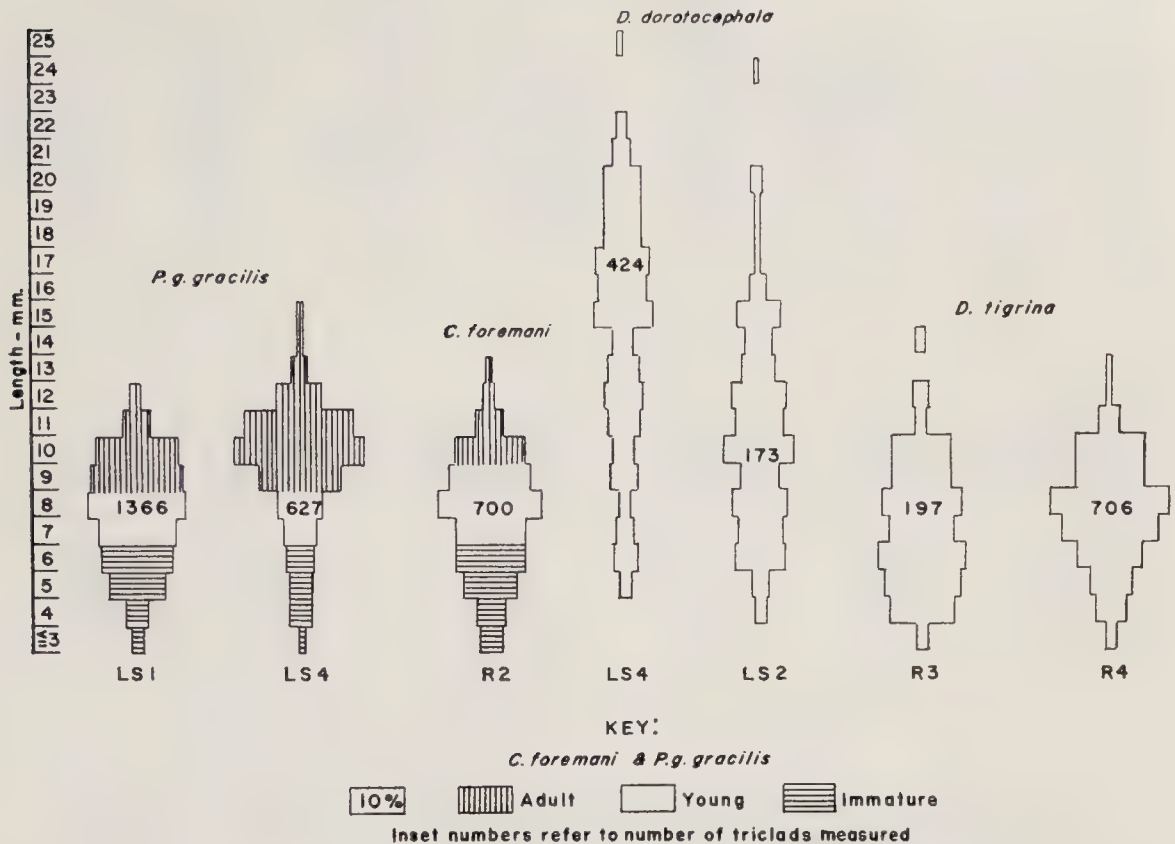


FIG. 13. Percentage size structure of the populations of *C. foremani*, *D. dorotocephala*, *D. tigrina*, and *P. g. gracilis* based on total numbers of triclads of each species collected at stations on Leonard Springs Branch and Richland Creek.

Monthly changes in size structure of the P. g. gracilis population at LS1. Monthly abundance indices indicate that drastic fluctuations in triclاد abundance did not occur at LS1 (Table 5), and an uninterrupted series of 13 monthly collections was possible at this station (Fig. 14). Hence, alterations in size structure throughout the year should be an approximate reflection of real changes within the population.

A general pattern of events can be recognized in Figure 14. In October and November 1963 there is a more or less uniform distribution of adults, young, and immatures. The percentage of adults (67%) increases in December, and the proportion of adults is relatively large from December through February. During this same period the percentage of immatures is low (11-16%). The percentage of adults decreases markedly between February and April and declines steadily to reach its lowest value (14%) in August. The percentage of immatures increases in July and August, and they comprise most of the population in September and October 1964.

In the absence of complete laboratory data on the breeding cycle of *P. g. gracilis* for comparison with field results, only general inferences can be drawn about changes in size structure. The pattern of events suggests that *P. g. gracilis* breeds and deposits egg capsules during winter and early spring when a relatively large number of adults is present.

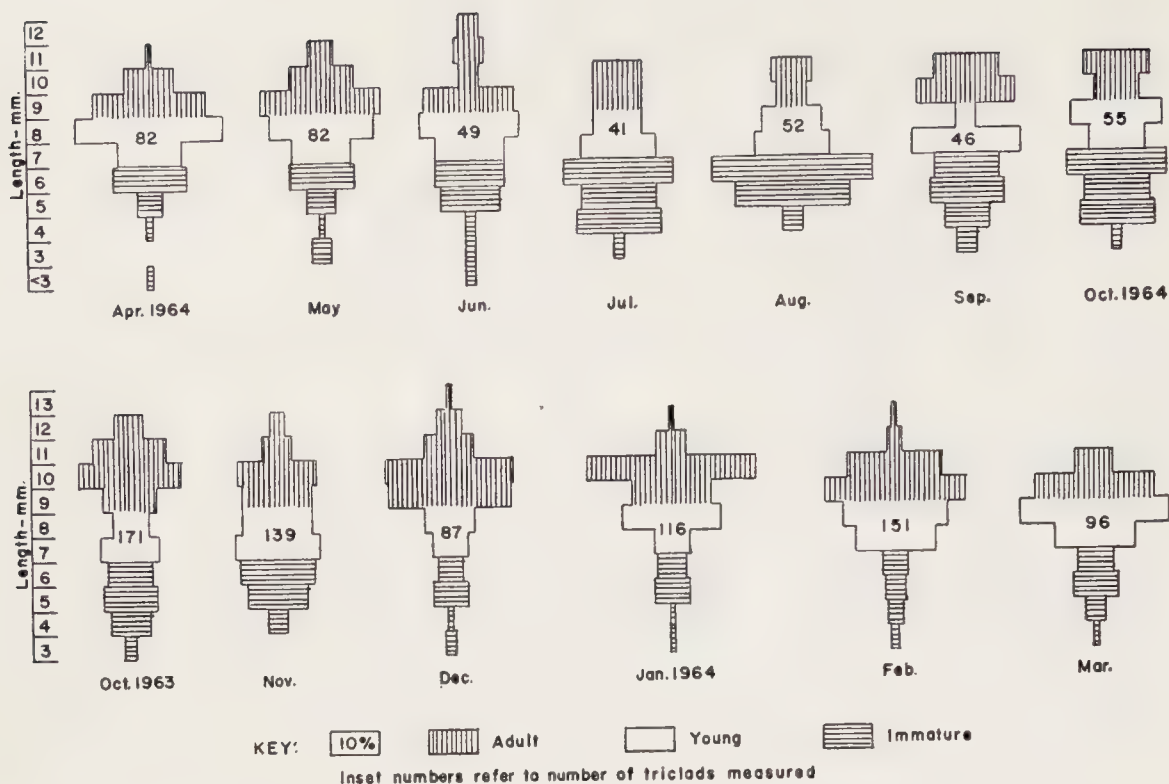


FIG. 14. Monthly changes in the percentage size structure of the population of *P. g. gracilis* at Station LS1 on Leonard Springs Branch

Some hatching may occur year round, but it appears to be most pronounced from April through August. From December through March the percentage of adults progressively decreases and coincides with a progressive increase in percentage of young. Apparently this increase in percentage of young is not entirely the result of recruitment of immatures into the young size group, since hatching appears to be most pronounced during April to August, and the percentage of immatures during December-March is about constant. Part of the increase in the young size group may be accounted for by the incorporation into the group of shorter adults. Shrinkage of these adults is probably a result of food shortage and/or increased activity during breeding. A similar phenomenon was reported by Reynoldson (1960, 1961) for two triclad species in England.

Relationship between D. dorotocephala and P. g. gracilis at Station LS4

As mentioned earlier, Station LS4 seems to be a transitional zone, since *D. dorotocephala* and *P. g. gracilis* occur here together; moreover, the former species is seldom found above LS4, whereas the latter apparently never occurs below LS4.

Collections of April through August 1964 at Station LS4 contained a greater percentage of *P. g. gracilis* (70-100%) than of *D. dorotocephala* (0-28%), indicating that the former species predominates during the spring and summer of 1964 (Fig. 15). In autumn of 1964 and winter of 1964-65 the reverse relationship obtains, i.e., *D. dorotocephala* becomes dominant, comprising from 74 to 97% of monthly collections, whereas

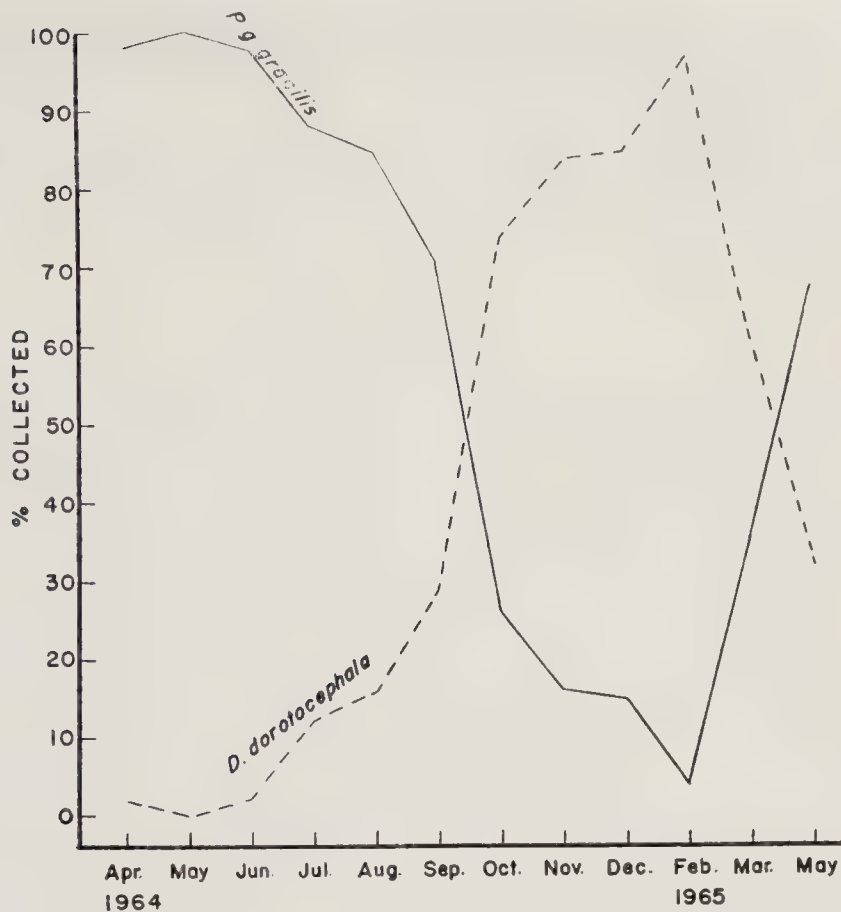


FIG. 15. Percentage of *D. dorotocephala* and *P. g. gracilis* in each monthly collection at Station LS4 on Leonard Springs Branch.

P. g. gracilis comprises only 4 to 26% of monthly collections during the same period. Moreover, in spring of 1965 there occurs a trend toward conditions found in spring of 1964, with *P. g. gracilis* being relatively more abundant (68%) than *D. dorotocephala* (32%).

The following explanation of this reciprocal relationship is suggested. The two species apparently have similar requirements as regards food and type of substrate, which would lead to some degree of interspecific competition. During spring and summer of 1964 *P. g. gracilis* out competes *D. dorotocephala*. However, in September of 1964, the abundance of *P. g. gracilis* in collections decreases markedly from 70 to 26%. This, I hypothesize, represents the migration of *P. g. gracilis* from station LS4 to areas nearer either Leonard Springs or Shirley Springs in order to breed during winter, thus allowing *D. dorotocephala* to dominate the transition zone. Two observations described earlier support this hypothesis: (1) the relatively low percentage of young and immature *P. g. gracilis* collected at LS4 implies that it breeds very little at this station (Fig. 13), and (2) annual changes in the size structure of the *P. g. gracilis* population at LS1 suggest that this species breeds mainly in winter at upstream areas nearer the headwaters (Fig. 14). In spring of 1965 large numbers of *P. g. gracilis* return to LS4 in order to feed, and this forces the majority of *D. dorotocephala* from the transition zone downstream.

Beauchamp (1933) observed similar migrational behavior in the European triclad *Planaria (Crenobia) alpina*, which migrates upstream to breed and downstream to feed.

The apparently competitive relationship at LS4 further suggests that *D. dorotocephala* may be prevented from inhabiting the headwater regions because of inability to compete with large numbers of *P. g. gracilis* in these areas.

Additional observations

Stalked capsules of *C. foremani* were found attached to stones at RIX and R2 throughout most of the sampling period, suggesting that breeding is quite successful at these two stations. However, no triclad capsules were found at R3, R4, and LS2. Efforts to find capsules of *P. g. gracilis* proved fruitless during the first 14 months of field work. In December of 1964, while collecting between LS1 and LS4, I noticed a triclad capsule lodged in a small depression on the undersurface of a stone that was partially covered with a 1-2 mm layer of loosely packed material apparently consisting largely of CaCO_3 and slit. Careful removal of pieces of this covering revealed 26 unstalked capsules, some being within the loosely packed material, while others were on the stone under the covering. Similar stones were then examined at LS1 and Shirley Springs, and 6 to 8 unstalked capsules were added to the original collection. Closer examination of these capsules revealed the following additional features: spherical; reddish-brown to almost black; leathery in texture, with no apparent markings; and 0.75 to 1.25 mm in diameter. Some of these capsules later hatched several *P. g. gracilis*.

Qualitative samples of the stone fauna were occasionally obtained from each station (Table 6). *Lirceus* occurred at all stations, but it seemed to be less abundant at LS2 and R3 than at other stations. Amphipods (*Crangonyx* and/or *Gammarus*) were not found at R3 or R4 and seemed to be very scarce at LS2. They were most abundant at LS4 and somewhat less abundant at LS1 and R2. *Simulium* larvae virtually covered the stones at LS1 and Shirley Springs during early spring. *Psephenus* seemed to be absent from the upstream stations of Leonard Springs Branch but occurred abundantly at LS2 and the downstream stations of Richland Creek.

The diversity and composition of invertebrates in Leonard Springs Branch and Richland Creek compares well with that of some other streams in this general area (Murray, 1938; Slack, 1955). Moreover, the stone fauna of the upper reaches of both streams is similar to that found in spring-fed headwaters (Ricker, 1934) and in streams of eroding substrata (Hynes, 1960). The presence of mayflies, stoneflies, caddisflies, megalopterans, and malacostracans seems to indicate that both streams are free of gross pollution, since these organisms more often occur in relatively clean water zones than in septic zones (Kolkwitz, 1950; Gaufin and Tarzwell, 1952). However, the presence of *Physa* and Tendipedidae in Richland Creek and their apparent absence from Leonard Springs Branch suggest that the former stream is slightly more polluted than the latter, since both these taxa are often characteristic of septic zones (Kolkwitz, 1950; Gaufin and Tarzwell, 1952). *Simulium* often develops

TABLE 6. A list of some representatives of the stone fauna of Leonard Springs Branch (LS) and Richland Creek (R) based on occasional qualitative sampling. (x = present in samples.)

	Stations						
	LS1	LS4	LS2	R1X	R2	R3	R4
Malacostraca							
<i>Crangonyx</i>	x	x					
<i>Gammarus</i>	x	x	x	x	x		
<i>Lirceus</i>	x	x	x	x	x	x	x
Plecoptera							
<i>Nemoura</i>	x		x				
Ephemeroptera							
<i>Baetis</i>	x	x					
<i>Epeorus</i>	x	x					
<i>Isonychia</i>			x	x	x	x	
<i>Stenonema</i>				x	x	x	x
Megaloptera							
<i>Nigronia</i>						x	
Trichoptera							
<i>Chimarra</i>						x	
<i>Cheumatopsyche</i>	x	x		x	x	x	
<i>Diplectrona</i>	x						
<i>Helicopsyche</i>			x			x	
<i>Hydropsyche</i>	x	x					
<i>Leptocella</i>			x				
<i>Rhyacophila</i>	x	x				x	x
Diptera							
<i>Pedicia</i>					x		
<i>Simulium</i>	x	x			x	x	x
Tendipedidae					x		x
<i>Tipula</i>					x		
Coleoptera							
<i>Hydroporus</i>							x
<i>Psephenus</i>			x		x	x	x
<i>Stenelmis</i>			x			x	x
<i>Tropisternus</i>							x
Gastropoda							
<i>Goniobasis</i>			x			x	x
<i>Physa</i>				x	x	x	
<i>Pleurocera</i>			x				

large populations in stony rapids of mildly polluted streams (Klein, 1962), which may be why they are so abundant at LS1 during spring. This pollutional evaluation of both streams is substantiated later from observations on chemical features.

Field observations indicate that *C. foremani*, *D. dorotocephala*, and *P. g. gracilis* feed upon amphipods and isopods, since these live crustaceans placed in collecting pans with flatworms were immediately swarmed over by numerous triclads, which showed all signs of feeding. Twice *D. dorotocephala* was observed feeding upon amphipods and isopods in the stream, and once a dead oligochaete at LS4 was covered with *D. dorotocephala* and *P. g. gracilis*.

ENVIRONMENTAL OBSERVATIONS ON TEMPERATURE,
CHEMICAL FEATURES, AND OTHER PHYSICAL FEATURES

Temperature

Observations are of two types: (1) those based on continuous temperature records from recording thermometers for one year at Station LS1 on Leonard Springs Branch and at Stations R2 and R3 on Richland Creek, and (2) those based on weekly readings of max-min thermometers at Stations LS4 and LS2 on Leonard Springs Branch and at Stations R1X and R4 on Richland Creek. The first type of observation gives a rather complete picture of temperature conditions at three stations throughout the year, whereas the second type, although being less complete, does yield information about seasonal temperature regimes at the other four stations. Each type of observation is presented separately, and the information from each is evaluated as it relates to the distribution and abundance of the triclads.

Observations based on continuous temperature records at Stations LS1, R2, and R3. When the annual patterns of daily fluctuations in temperature (°C) of the three stations and of air are compared, it is apparent that LS1 has a pattern distinctly different from that of air or of R2 and R3 (Fig. 16). The latter two stations have patterns that coincide more closely with that of air than LS1. However, the degree of coincidence is greater for R3 than for R2. These differences become further apparent when the seasonal mean and annual mean temperature fluctuations of the air and of the three stations are compared (Table 7). A seasonal mean temperature fluctuation represents the average difference between daily maximum and minimum temperatures for a particular season. The annual mean temperature fluctuation is the average of the four seasonal mean temperature fluctuations, and hence it represents the average differences between the daily maximum and minimum temperatures for the entire year. There is little difference in the mean temperature fluctuation of each season at LS1, and all seasons show less

TABLE 7. Seasonal mean temperature fluctuation and annual mean temperature fluctuation (°C) of the air and of Station LS1 on Leonard Springs Branch and Stations R2 and R3 on Richland Creek. Seasonal periods determined according to equinoctial and solstitial times. Stations for Richland Creek are listed in order downstream.

Station	Seasonal mean fluctuation				Annual mean fluctuation
	Autumn	Winter	Spring	Summer	
LS1	2.0	1.5	1.0	1.5	1.5
R2	5.8	3.0	3.8	6.4	4.8
R3	5.8	2.2	7.3	7.4	5.7
Air	11.6	9.4	10.6	11.9	10.9

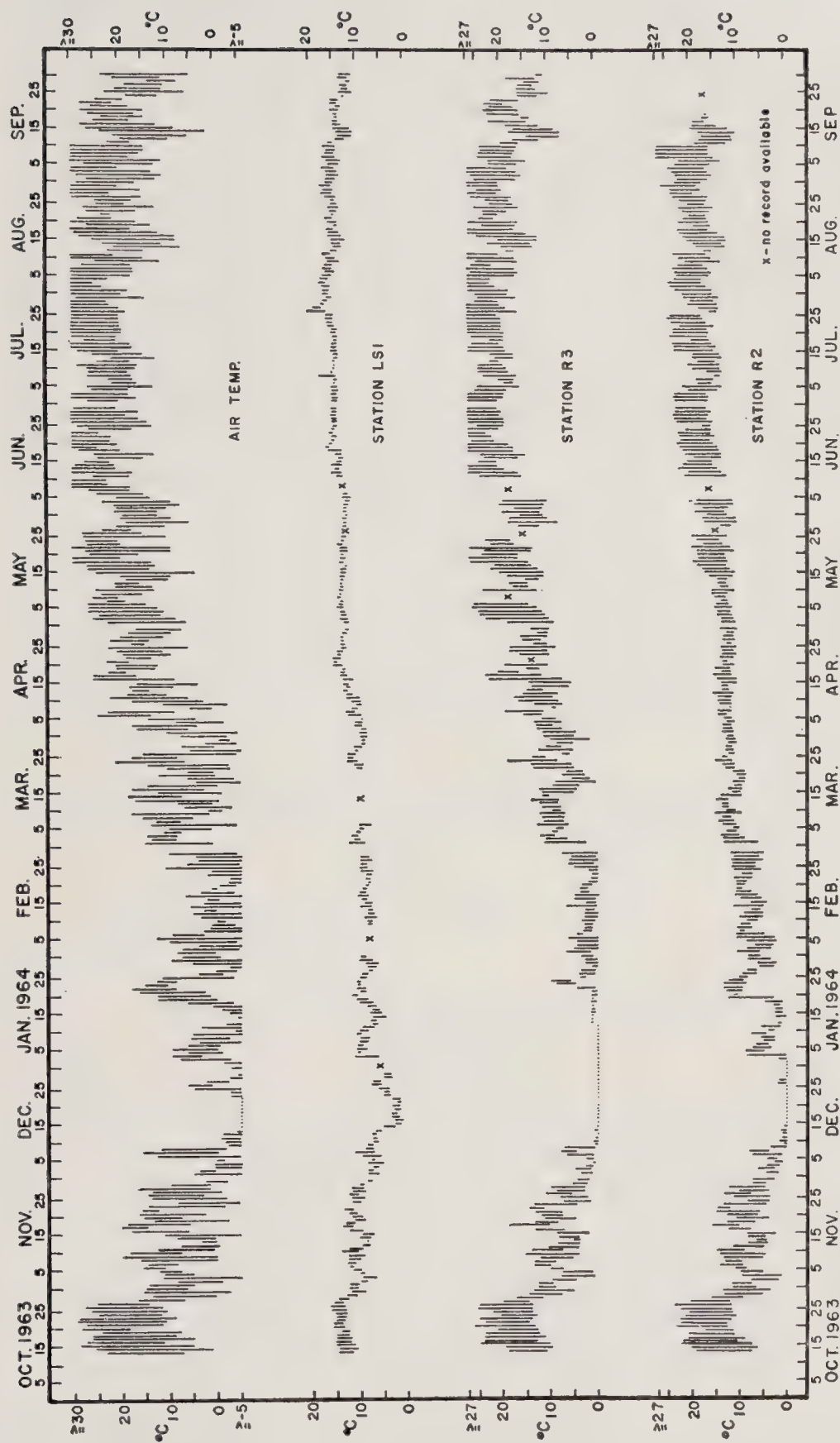


Fig. 16. Daily max-min water temperatures at Station LSI on Leonard Springs Branch and at Stations R2 and R3 on Richland Creek, based on continuous temperature records from recording thermometers, and daily max-min air temperatures as recorded at the Bloomington Airport. For the latter the symbol $\geq 5^{\circ}\text{C}$ denotes that the temperatures were equal to or lower than 5°C .

fluctuation than either R2 or R3 (Table 7). Seasonal mean temperature fluctuations at R2 and R3 are somewhat similar except during spring, when fluctuation at R2 (3.8°C) is considerably less than at R3 (7.3°C). Each of stations R2 and R3 has an annual mean temperature fluctuation more than three times greater than that of LS1, and that of the air is roughly seven times greater than LS1.

Daily temperature extremes of 0°C and $\geq 27^\circ\text{C}$ occurring at R2 and R3 during winter and summer, respectively, do not occur at LS1, where they are 1.1°C and 20.6°C, respectively (Fig. 16). Moreover, these winter and summer extremes occur much more often at R2 and R3 than at LS1. During December and January the water temperature at LS1 never reached 0°C, although air temperatures of -5°C or lower ($\geq -5^\circ\text{C}$) occurred on 37 days during these months. Air temperatures of $\geq 30^\circ\text{C}$ occurred on 43 days during summer, but daily water temperatures at LS1 rarely exceeded 18°C during this season. Comparison of daily readings of 0°C during December-February at R2 and R3 shows that these occurred on 28 days at the upstream station but on 51 days at the downstream station, and similarly, temperatures of $\geq 27^\circ\text{C}$ during May-September occurred on only 4 days at R2 but on 46 days at R3.

The highest air temperature (36.7°C) was recorded on 3 August 1964, the lowest for this day being 25.6°C. The daily cycles of water temperature at the three stations on this date contrast decidedly (Fig. 17). Variation of temperature is least at LS1, greatest at R3, and somewhat intermediate at R2. The daily mean temperature at R3 (27.5°C) is considerably greater than at LS1 (17.4°C) or R2 (21.4°C). Moreover, dura-

TABLE 8. Thermal relationships and differences in water temperature (°C) at Station LS1 on Leonard Springs Branch and at Stations R2 and R3 on Richland Creek, based on the daily cycles of temperature shown in Figure 17.

	Stations		
	LS1	R2	R3
Max. temp.	18.1	25.0	32.2*
Time max. occurred	1500	1500	1200
Previous min. temp.	16.7	17.8	22.8
Time min. occurred	1030	0600	0600
Difference in max. and previous min.	1.4	7.2	9.4
Time required to go from previous min. to max. (hrs)	4.5	9.0	6.0
Mean rate of heating (°C/hr)	0.31	0.80	1.56
Time required to go from max. to min. equal to previous min. (hrs)	4.5	12.0	12.0
Mean rate of cooling (°C/hr)	0.31	0.60	0.78

* Extrapolated from recording thermometer chart

tion of the maximum temperature is different at the three stations, being 1.5 hours at 18.1°C for LS1, 3 hrs at 25°C for R2, and 4.5 hrs at 32.5°C for R3. A more detailed contrast of the daily cycles shown in Figure 17 reveals that the thermal relationships and differences in water temperature at the three stations are not the same (Table 8). The upstream stations LS1 and R2 each have a lower maximum and minimum temperature, lower mean rates of heating and cooling, and less temperature fluctuation than the downstream station R3.

The greatest fluctuation in air temperature occurred on 14 October 1963, being 24.5°C in a range of 1.1—25.6°C. Comparison of fluctuations in water temperature at the three stations on this date (Table 9) shows that LS1 had the least fluctuation, with R2 and R3 having greater fluctuations, which are similar.

Examination of mean water temperatures for each season at the three stations results in a comparison similar to those above (Table 10). A seasonal mean represents the average of all daily means for a particular season. Seasonal means, standard errors, and N values for the three

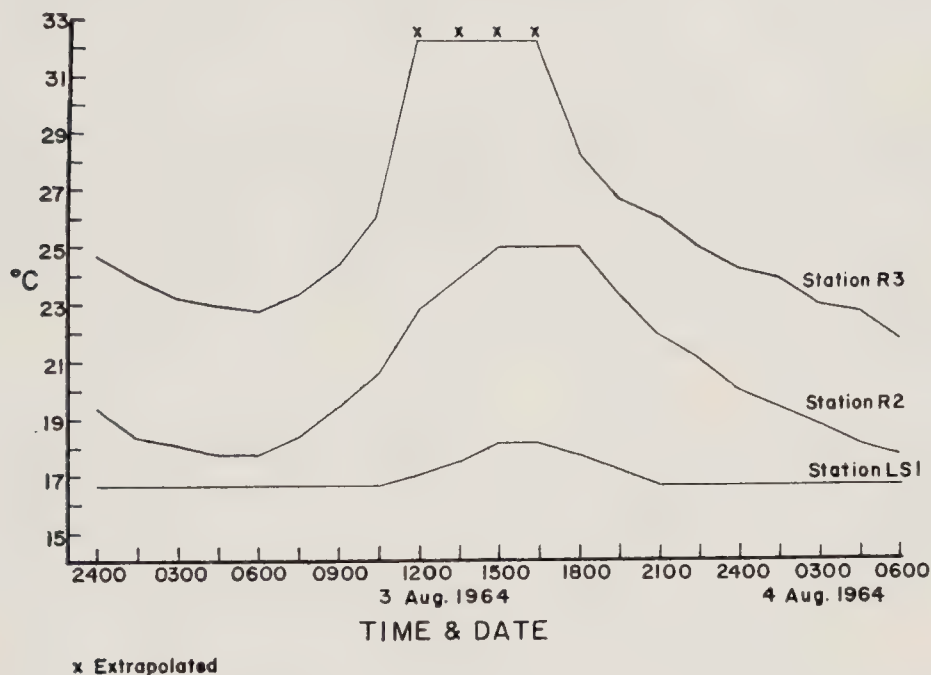


FIG. 17. Fluctuations in water temperature from 2400 hours on 2 Aug. 1964 to 0600 on 4 Aug. at Station LS1 on Leonard Springs Branch and at Stations R2 and R3 on Richland Creek. Temperatures at 1.5-hour intervals were read from continuous records on circular, 7-day charts graduated in 3-hour intervals and with a range of from -6.7 to 26.7°C. For Station R3, which considerably exceeded the above average, some of the higher values could be extrapolated up to the maximum excursion of the recording arm. Interpolation and extrapolation are most difficult at higher temperatures toward the center of the chart, because here the 3-hour grid lines are very close together. Hence, the limited range of the instrument, the potentially large errors of extrapolation at the high temperatures, and the fact that the temperatures are plotted only at 1.5-hour intervals probably accounts for the peculiar truncated peaks, especially of R3. The absolute shape of the curves, however, is less important than the relative differences between the stations. On 3 Aug. the air temperature ranged from 25.6 to 36.7°C.

TABLE 9. Maximum and minimum temperatures (°C), temperature fluctuations (°C), and fluctuation ratios of water temperature to air temperature at Station LS1 on Leonard Springs Branch and Stations R2 and R3 on Richland Creek for 14 October 1963 when air temperature fluctuated a total of 24.5°C between 1.1°C and 25.6°C.

	Stations		
	LS1	R2	R3
Max. temp.	14.4	20.0	21.1
Min. temp.	10.6	6.1	7.8
Fluctuation	3.8	13.9	13.3
Fluctuation ratio of water temp. to air temp.	1:6.4	1:1.8	1:1.8

stations are shown in Table 10. Seasonal mean temperatures vary least at LS1 and most at R3, with R2 being somewhat intermediate. Comparison of the mean values for summer and winter indicates that LS1 is considerably cooler in summer (15.4°C) and warmer in winter (8.2°C) than either R2 or R3 and that of the latter two stations, R2 is cooler in summer (18.7°C) and warmer in winter (6.8°C) than R3 (20.7°C and 3.0°C, respectively). Application of the “*t* test” (Moroney, 1953) showed that any two stations being compared differ significantly (above 0.1% level) in their means for spring, summer, and winter. Differences between autumn means of Stations LS1 and R2 are significant at the 1% level, and between LS1 and R3 at the 5% level. The autumn difference between R2 and R3 is not significant.

TABLE 10. Seasonal mean temperatures (averages of daily means), standard errors, and numbers of observations (N) at Station LS1 on Leonard Springs Branch and at Stations R2 and R3 on Richland Creek. Seasonal periods were determined according to equinoctial and solstitial times. For Richland Creek the stations are listed in order downstream.

Season	Station	Seasonal mean temp. (°C)	Standard error	N
Autumn	LS1	9.9	± 0.6	136
	R2	8.2	± 0.6	138
	R3	8.4	± 0.6	138
Winter	LS1	8.2	± 0.2	128
	R2	6.8	± 0.3	180
	R3	3.0	± 0.3	180
Spring	LS1	12.8	± 0.1	170
	R2	9.2	± 0.4	170
	R3	14.8	± 0.4	158
Summer	LS1	15.4	± 0.1	188
	R2	18.7	± 0.3	178
	R3	20.7	± 0.3	204

Observations based on weekly readings of max-min thermometers at Stations LS4, LS2, R1X, and R4. A general idea of temperature regimes can be formulated by examining the mean temperatures, the mean temperature fluctuations, and the highest and lowest weekly temperatures at each station for each season (Table 11). Seasonal mean temperatures were obtained from the weekly means, and comparison of these indicates that in both streams the upstream stations (LS4 and R1X) are distinctly warmer in winter and cooler in summer than the downstream stations (LS2 and R4). Seasonal mean fluctuations were obtained by averaging the difference between max-min readings for each season at a particular station. The low numbers of weekly observations for some seasons result from the difficulty encountered in maintaining max-min thermometers at the stations. The upstream stations, LS4 and R1X, show less mean fluctuations for autumn, spring, and summer than the downstream stations LS2 and R4 (Table 11). The mean fluctuations for winter are somewhat greater at the upstream than at the downstream stations, suggesting that warming trends occur more often at the former stations. All stations except LS4 have weekly minimum temperatures of 0°C in winter, and although these occur at LS2 and R4 in autumn, they do not

TABLE 11. Mean temperatures (°C), mean temperature fluctuations (°C), highest weekly maximum and lowest weekly minimum temperatures (°C), and numbers of weekly observations for each season at Stations LS4 and LS2 on Leonard Springs Branch and at Stations R1X and R4 on Richland Creek. R1X values include R1 observations. For each stream the stations are listed in order downstream. Seasonal periods were determined according to equinoctial and solstitial times.

Season	Station	Seasonal mean temp.	Seasonal mean fluctuation	Highest weekly maximum	Lowest weekly minimum	No. of weekly observations
Autumn	LS4	10.3	7.6	16.7	3.3	13
	LS2	8.0	9.2	23.3	0.0	10
	R1X	12.3	4.2	20.0	4.4	10
	R4	9.1	7.7	23.9	0.0	10
Winter	LS4	8.2	6.2	12.7	2.7	5
	LS2	4.0	5.7	13.3	0.0	12
	R1X	7.5	5.4	12.2	0.0	12
	R4	4.9	4.7	14.4	0.0	12
Spring	LS4	14.5	3.7	17.8	10.0	10
	LS2	12.9	7.8	26.7	3.9	3
	R1X	12.4	3.0	18.9	10.0	10
	R4	13.7	7.6	27.8	2.8	7
Summer	LS4	16.7	3.8	22.8	13.3	10
	LS2	20.4	12.3	31.1	10.6	7
	R1X	16.5	7.8	23.3	12.2	12
	R4	21.3	9.3	32.2	8.9	12



FIG. 18. Temperature regimes and triclad distribution and abundance in Leonard Springs Branch.

occur at LS4 or R1X. Moreover, the highest weekly maximum temperatures for autumn, spring, and summer are considerably less at LS4 and R1X than at LS2 and R4 (Table 11).

Evaluation of temperature observations. The thermal environment of LS1 is milder and less erratic than that of either R2 or R3 in terms of daily, seasonal, and annual fluctuations in water temperature. Station R3 has a harsher and more erratic thermal environment than R2. Similarly, greater extremes of temperature occur at R2 and R3 than at LS1, but they are of lesser magnitude and duration at R2 than at R3.

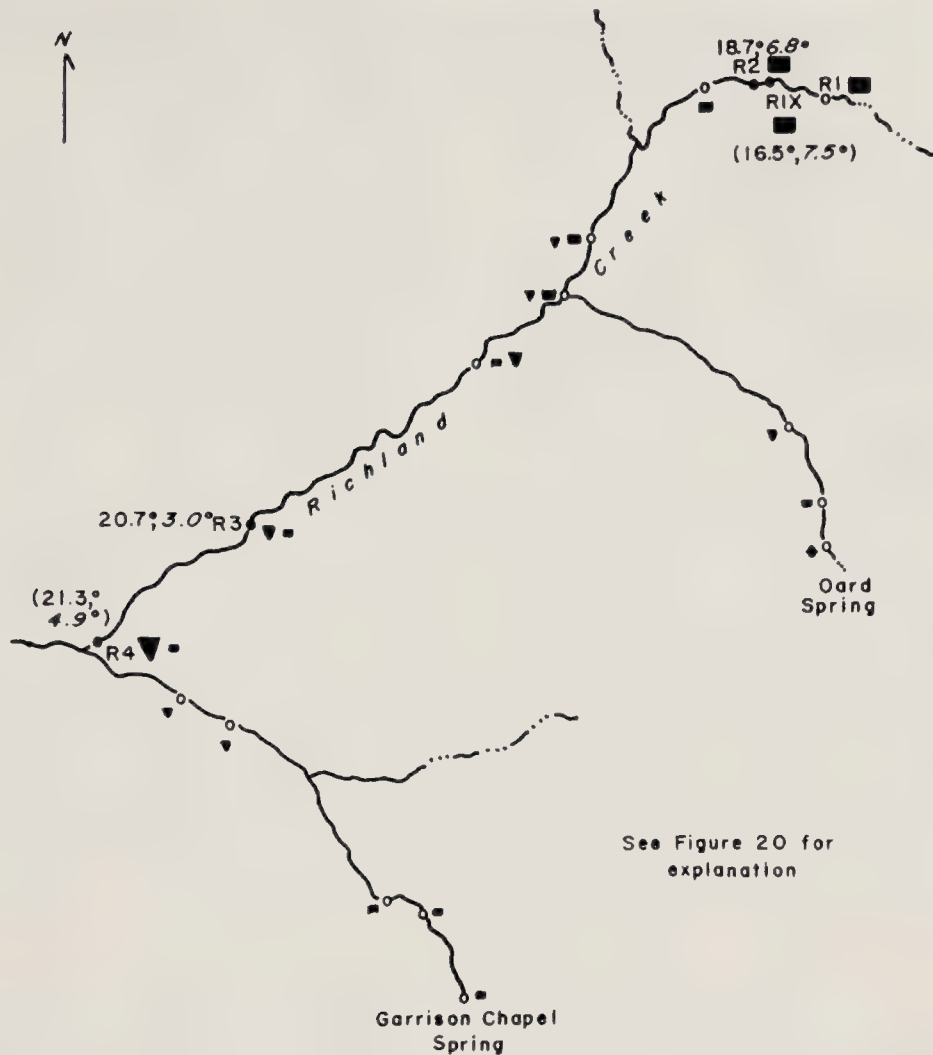


FIG. 19. Temperature regimes and triclad distribution and abundance in the upper reaches of Richland Creek.

These contrasting temperature regimes seem directly related to triclad distribution and abundance (Fig. 18, 19). The abundance of *P. g. gracilis* at LS1 (Fig. 18) suggests that it is quite successful in the unusually mild temperature regime of this station. *C. foremani* is scarce at R3 (Fig. 19) but is abundant in the comparatively less severe thermal environment of R2.

The temperature regimes of the upstream stations having max-min thermometers (LS4 and R1X) are similar to those of LS1 and R2 (Fig. 18, 19), both showing lower summer and higher winter mean temperatures. Moreover, they exhibit less difference in these means than the downstream stations (LS2, R3, and R4).

P. g. gracilis is abundant at LS4 but never occurs at LS2 (Fig. 18) where comparatively greater temperature fluctuations and warmer mean summer temperatures prevail, and *D. dorotocephala* is more abundant at LS4 than at LS2 (Fig. 18). This suggests that temperature is affecting the distribution of both species, but to a somewhat greater extent for *P. g. gracilis*. The only abundant triclad at R4 is *D. tigrina* (Fig. 19),

and its abundance decreases upstream, finally being absent from R1, R1X, and R2. This suggests that *D. tigrina* is well adapted to the warm summer temperatures of Stations R3 and R4.

Hynes (1960) refers to three factors that influence water temperature: source of water, local surroundings (aspect), and altitude. Since all stations have essentially the same altitude, this factor can be neglected.

Source of water is very important. Station LS1, being close to the rheokrene source of Leonard Springs Branch, reflects more nearly the mean annual air temperature of the region (11.1°C) than any other station in either stream. Although LS4 is downstream from LS1, it is still influenced more by source temperature than by local weather conditions. The limnokrene at R1X exhibits about the same annual temperature fluctuation as LS1 and supplies most of the flow at R2. The water temperature of R2 likewise reflects mostly the influence of the source rather than of surface runoff. Surface runoff sustains most of the flow at Stations LS2, R3, and R4, and water temperatures reflect local weather conditions prevailing at the time.

Shading is an important factor regulating water temperature, because direct radiation from the sun is more effective in warming the water than is mere contact with warmer air (Ricker, 1934). As noted in Table 1, shading is extensive at LS1 and moderate at LS4, R1X, and R2. The lack of any appreciable shading at LS2, R3, and R4 undoubtedly affects the temperature regimes of these stations. The differential effect of shading is clearly evident from the cycles of temperature at LS1, R2, and R3 during a hot summer day (Fig. 17, Table 8).

Chemical features and other physical features

These observations were made in order to define more fully the environmental conditions of each station and to detect any important differences in these conditions that might affect the distribution and abundance of triclads. The presentation of results emphasizes seasonal variation of some chemical and physical parameters at each station (Fig. 20) and horizontal variation of the same parameters during periods of normal and high water levels (Fig. 21). The former aids in describing changes in the environment to which triclads are subjected throughout the year, and the latter gives some idea of the extremes, since most of the parameters are correlated with changes in water level. Additional observations, not amenable for inclusion in the above figures, are reported in tabular form or otherwise described.

Values plotted in Figure 20 are seasonal means, each obtained by averaging from 4 to 14 observations. For each parameter in Figure 20, the mean annual value and extreme values at each station are shown in Table 12. Observations began in the winter of 1964 at station R1X and in the spring of 1964 at LS4. Turbidity observations began in the winter of 1964.

Figure 21 compares values for each parameter during high water level (5 March 1964) and normal water level (15 February 1964). The Bloomington airport recorded 2.82 inches of precipitation between 15 February and 5 March 1964, 2.43 inches of this occurring on 2, 3, and

TABLE 12. Annual mean values and extreme values of some chemical and physical parameters at all stations on Leonard Springs Branch (LS) and Richland Creek (R). For both streams the stations are listed in order downstream.

	Sta.	Annual mean	Extremes	
			High	Low
Calcium (ppm)	LS1	69	98	31
	LS4	65	89	39
	LS2	65	75	47
	R1X	91	102	64
	R2	89	122	63
	R3	74	102	51
	R4	77	109	57
Chloride (ppm)	LS1	11.0	25	3.1
	LS4	7.0	15	3.0
	LS2	8.0	16	3.7
	R1X	7.3	11	5.0
	R2	7.1	13	4.6
	R3	12.0	23	4.9
	R4	11.0	20	4.8
Dis- solved organic matter (mgO ₂ /l absorbed from KMnO ₄)	LS1	3	9	0
	LS4	3	8	1
	LS2	2	8	1
	R1X	3	10	1
	R2	4	10	1
	R3	3	9	1
	R4	3	8	1
Hardness (ppm Ca plus Mg)	LS1	76	101	35
	LS4	73	103	45
	LS2	72	87	50
	R1X	99	113	67
	R2	97	123	67
	R3	85	113	62
	R4	87	116	61
Mag- nesium (ppm)	LS1	6.8	13	1.5
	LS4	8.3	15	3.2
	LS2	7.8	13	3.7
	R1X	7.4	19	3.5
	R2	8.3	17	0.6
	R3	11.0	17	3.2
	R4	9.2	16	3.3
Specific conduct- ance (μ mhos at 18°C)	LS1	75	134	28
	LS4	72	114	35
	LS2	66	91	48
	R1X	92	162	52
	R2	96	150	52
	R3	87	136	51
	R4	87	150	50
Turbid- ity (ppm SiO ₂)	LS1	15	85	2
	LS4	10	70	2
	LS2	10	55	4
	R1X	9	35	3
	R2	8	40	2
	R3	7	35	1
	R4	10	35	2
Water level (inches)	LS1	7	13	4
	LS4	—	—	—
	LS2	9	24	4
	R1X	—	—	—
	R2	5	11	4
	R3	10	19	6
	R4	12	21	8

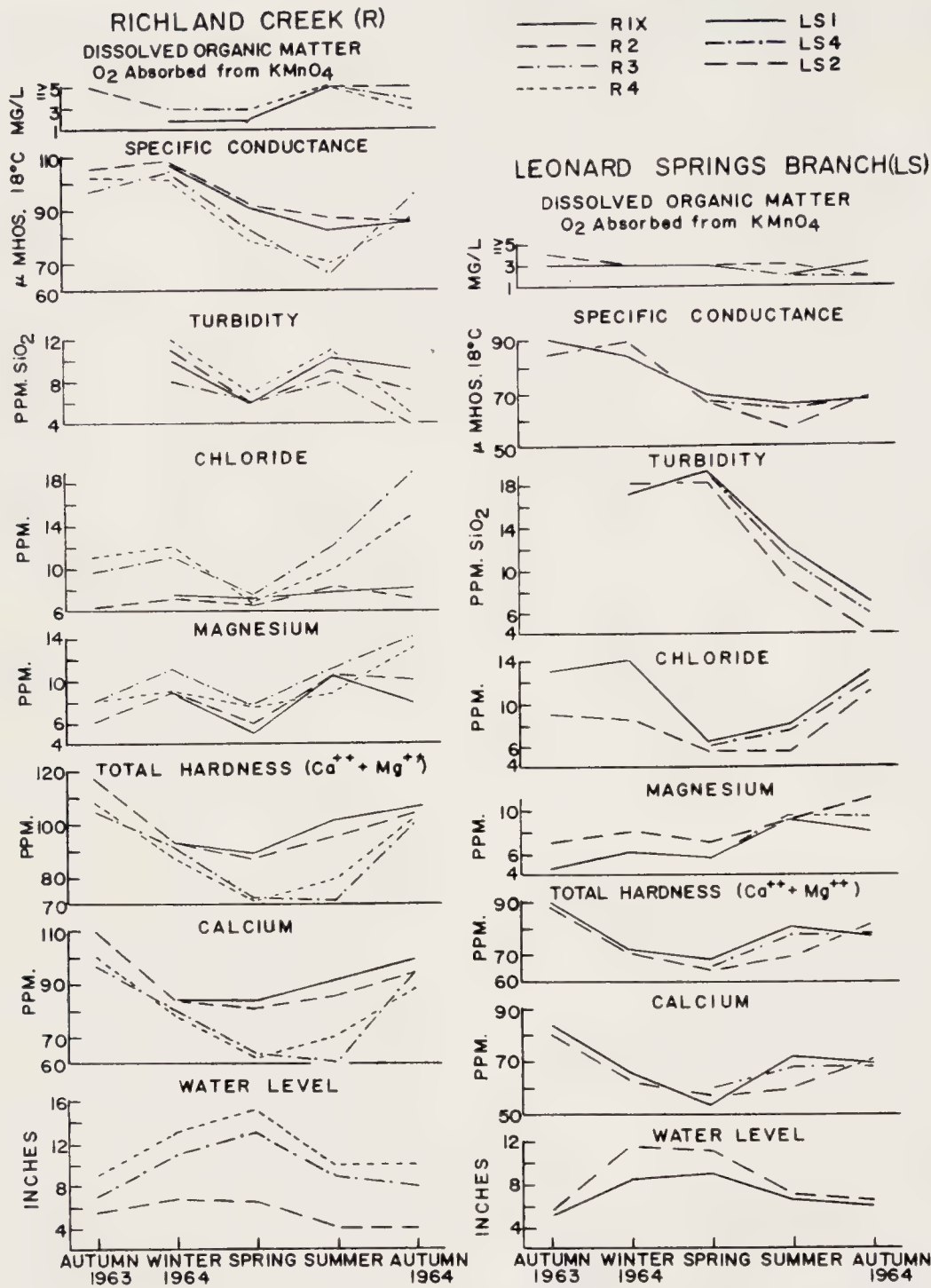


FIG. 20. Seasonal variation of some chemical and physical parameters at stations on Leonard Springs Branch and Richland Creek.

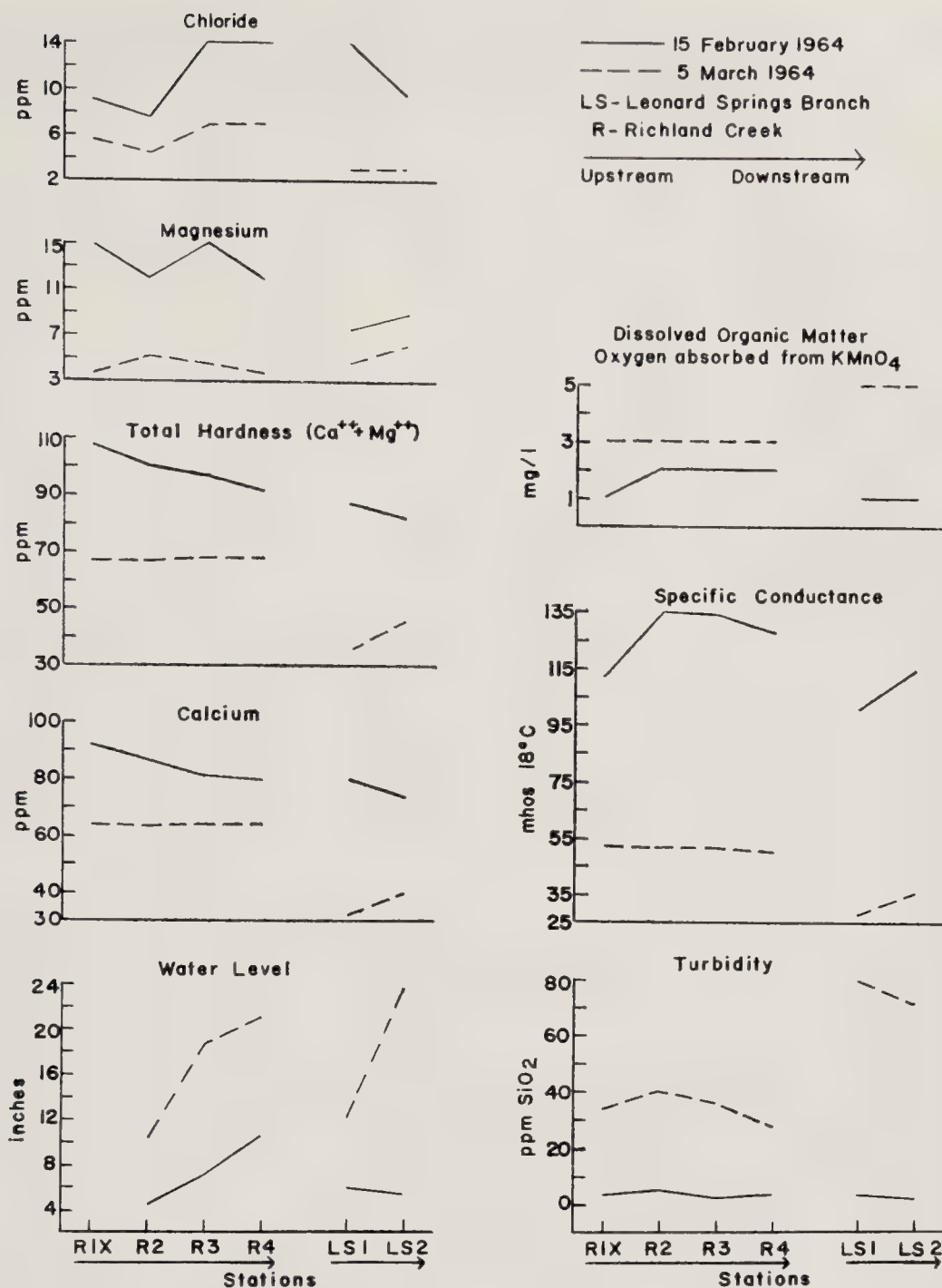


FIG. 21. Horizontal variation of some chemical and physical parameters on Leonard Springs Branch and Richland Creek on a day of high water level (5 Mar. 1964) and a day of normal water level (15 Feb. 1964).

4 March. All stations were visited on 5 March 1964, about 16 hours after cessation of the heavy rains. Conditions of discharge in both streams contrasted remarkably with those that normally prevail. Torrential conditions existed at all stations and were particularly impressive at Station LS1.

Water level and volume of flow: Volume of flow was measured infrequently, but water level was recorded weekly. As explained under methods, water level approximates "water depth," and, as expected, there is some correlation between water level and volume of flow (discharge) at each station (Table 13).

Water levels are highest in both streams during winter and spring, with low levels occurring in autumn and summer (Fig. 20). The winter of 1964 was actually a season of low to moderate levels, and hence the high values of Figure 20 reflect mainly the heavy rains during the first part of March. The period prior to the autumn of 1963 was one of very little precipitation, and although no discharge data are available for this period, water levels were consistently low.

Difference in discharge is greater between the downstream and upstream stations on Richland Creek than between those on Leonard Springs Branch, being much higher at R3 and R4 than at R2 from winter to autumn of 1964. However, station LS2 exhibits the highest discharge for any single week, and LS1 shows a higher discharge on 5 March 1964 than R1X (Fig. 21). The apparent correlation between high discharge and relative abundance of triclads at some of the stations is brought out in the discussion section.

On a day of normal flow Leonard Springs Branch shows little horizontal variation from source to mouth compared to Richland Creek (Fig. 21). The flow from tributaries entering Richland Creek below R2 accounts for most of the difference in discharge. The tributary from Oard Spring (Fig. 4) contributes considerable water to Richland Creek, especially during heavy rains, as evidenced by the sharp rise in water level at R3 (Fig. 21). Tributary flow is less important in Leonard Springs Branch during periods of normal precipitation. However, during heavy rains, formerly dry rivulets become charged with surface runoff, and volume of discharge increases suddenly below LS1.

Stream flow was uninterrupted at all stations except R3 and R4 throughout the observation period. However, at these stations such conditions prevailed for only short periods. Riffle areas became dry, and isolated pools existed at R4 on 5 and 12 September 1964 and again on 3, 10, and 16 October 1964. Small riffles occurred in the immediate vicinity of R3 on 5 and 12 September 1964. Thus, for most of the time planarians at all stations are not exposed to desiccation, which would probably substantially decrease their abundance, since their only recourse for survival under such conditions is to burrow into the stream bottom (Hynes, 1958).

TABLE 13 Relationship between discharge (volume of flow in ft³ per sec.) and water level (in.) at stations on Leonard Springs Branch (LS) and Richland Creek (R). For each stream the stations are listed in order downstream.

Station	15 Feb. 1964		29 Feb. 1964		28 March 1964	
	Discharge	Water level	Discharge	Water level	Discharge	Water level
LS1	1.7	6.0	1.2	6.5	5.7	11.0
LS2	1.3	5.5	1.4	5.5	12.5	13.0
R2	0.3	4.5	0.4	5.5	4.5	10.0
R3	1.0	7.0	1.3	7.5	9.1	13.5
R4	1.2	10.5	1.8	11.0	17.8	17.0

Calcium, total hardness, and magnesium: Total hardness is dominated by calcium, with magnesium playing a relatively minor role. Since magnesium is determined by differences between the titrations for calcium and for total hardness, its lack of trend relative to the other two variables may be a function in part of end-point errors. Concentrations of calcium and total hardness vary inversely with water level and decrease progressively downstream (Fig. 20), which results from dilution by the higher discharge downstream than upstream. The mean concentrations of calcium, magnesium, and total hardness are higher in Richland Creek than in Leonard Springs Branch (Table 12). When the mean value of calcium in each stream is multiplied by 2 497 (Hem, 1959), the results show a mean hardness (ppm CaCO₃) of 165 for Leonard Springs Branch and 207 for Richland Creek. Relating these values to the classification of Klein (1957), Leonard Springs Branch would be considered "moderately hard" and Richland Creek, "hard." The ratio of Ca to Mg in terms of equivalent parts per million is about 5 to 1, indicating that relatively pure limestone or other CaCO₃ precipitates dominate in the drainage area. The high calcium content of both streams indicates that they are stable chemically, which is substantiated by the observation that each stream maintains a rather constant pH (7.2-7.6) throughout the year. Generally, hard-water streams support a more diversified and abundant fauna than soft-water streams (Hynes, 1960), and some planarians are known to favor hard waters (Macan, 1963). The faunistic evaluation of Leonard Springs Branch and Richland Creek seems to support both these statements.

Comparison of headwater stations in the two streams reveals that the mean annual calcium content is 22 ppm less at LS1 than at R1X (Table 12). Moreover, mean seasonal values for Ca are less at LS1 (Fig. 20). Part of this difference in Ca content is apparently because of the loss of equilibrium CO₂ from the water as it emerges from Shirley Springs, and this accounts for the encrustation of CaCO₃ on the stones in the upper reaches of Leonard Springs Branch. The importance of this condition for *P. g. gracilis* is discussed later.

Chloride: Mean seasonal values of this ion are much higher at Stations R3 and R4 than at R1X and R2, except during high water levels in spring when they are relatively low at all stations (Fig. 20). Moreover, R1X and R2 show very little variations of chloride content throughout the year. Stations on Leonard Springs Branch have similar chloride values for spring, summer, and autumn of 1964, but during autumn of 1963 and winter of 1964 these values are much higher at LS1 than at LS4 (Fig. 20).

Increased amounts of chloride generally suggest that organic matter of animal origin is entering the stream and is being oxidized (Klein, 1957; Taylor, 1958). The animal excreta from three cattle farms along Richland Creek between Stations R2 and R4 account for most of the higher chloride at R3 and R4. Some of the chloride probably enters Richland Creek via the Oard Spring tributary, which receives CaCl_2 waste from a limestone quarry. Land used largely for pasturing cattle surrounds the headwaters of Leonard Springs Branch, and numerous swallow holes funnel surface runoff into the underground springs. Thus most of the high chloride at LS1 probably arises from animal excreta in the catchment basin.

Dissolved organic matter: As explained under "Methods," the sample volume for each analysis was that recommended by Mackereth (1963) for unpolluted water. Under such conditions, any value of 5 mg O_2/l or greater (Figs. 20 and 21) probably indicates pollution but does not reflect the absolute amount of dissolved organic matter or the extent of pollution. Biweekly values of 5 mg O_2/l or greater were recorded about 27% of the time in Richland Creek and about 9% of the time in Leonard Springs Branch, suggesting that the former stream may be occasionally more polluted than the latter. However, biweekly values less than 5 mg O_2/l were predominant, suggesting that both streams are relatively unpolluted over most of the year. This evaluation agrees with the earlier assessment of pollution, which was made on the basis of diversity and composition of the invertebrate fauna in the two streams.

Dissolved organic matter tends to vary inversely with water level in terms of seasonal means (Fig. 20) but exhibits the opposite relationship for particular days (Fig. 21). A sudden increase in dissolved organic matter accompanies an increase in stream discharge at all stations, especially in Leonard Springs Branch (Fig. 21), probably because of a sudden influx of animal excreta from barnyards and septic tanks.

Dissolved oxygen: Field determinations of dissolved oxygen were made twice during the summer of 1964, at times when low water levels and high temperatures might be expected to produce low dissolved oxygen values. Water samples were obtained from pooled areas on both occasions, except at Shirley Springs and R1X where samples were taken from the emerging spring water. All stations, except R1X for 3 July 1964, exhibit relatively high values of dissolved oxygen and per cent saturation (Table 14). Super saturations occurring at some of the downstream stations result from a combination of biological and physical conditions. Shading is not extensive at the downstream stations, and this favors a large production of oxygen by aquatic plants. This excess oxygen is not easily exchanged with the air, since the water is more sluggish downstream than upstream.

TABLE 14 Dissolved oxygen and percentage saturation values on two dates of low water levels in Leonard Springs Branch (LS) and Richland Creek (R).

Station	3 July 1964		14 August 1964	
	mgO ₂ /l	% sat.	mgO ₂ /l	% sat.
Shirley Springs	9.8	92	10.2	97
LS1	9.9	98	9.9	95
LS4	10.1	100	10.7	102
LS2	9.6	111	10.0	99
R1X	5.9	55	8.3	84
R2	6.8	76	8.2	87
R3	6.9	84	11.4	127
R4	7.5	89	10.7	118

There seems little indication from these results that dissolved oxygen might at any time drop to levels so low as to be a serious limiting factor for planarians.

Specific conductance: Mean values were higher in both streams during autumn of 1963 and winter of 1964 (Fig. 20). Reference was made to the low water levels that prevailed prior to and during autumn of 1963 and winter of 1964. Under these conditions the concentration of total dissolved solids increases and is reflected in higher conductance values. A sharp decrease in specific conductance coincides with consistently higher water levels in spring (Fig. 20). The dilution effect on specific conductance is clearly shown in Figure 21.

Turbidity: The two streams are obviously different in seasonal variation of turbidity (Fig. 20). For Leonard Springs Branch, turbidity varies directly with water level, whereas for Richland Creek there appears to be an inverse relationship. Consideration of the nature of the water supply (source water or surface runoff) of each stream helps to explain some of the difference in turbidity curves. A large part of the water supply to Leonard Springs Branch must pass through subterranean channels before emerging at Shirley Springs, and the water becomes charged with a large amount of suspended solids from these channels during periods of appreciable rainfall. Constant turbulence in the upper reaches of Leonard Springs Branch keeps particles suspended in the water for some distance downstream. On the other hand, surface runoff accounts for most of the flow in Richland Creek, and any turbulence produced soon abates. Thus turbidity in Richland Creek is less and lasts for a shorter time. Heavy rain, preceded by a dry period, produces considerable turbidity in both streams (Fig. 21). However, turbidity behaves quite differently in the two streams during periods of light to moderate rainfall such as that recorded for 15 days between 21 March and 22 April 1964. The relationship between this type of rainfall distribution and turbidity is compared for both streams in Table 15 and aids in further explaining the difference in turbidity patterns.

The difference in turbidity values between the two streams for summer and autumn cannot be accounted for in the same way. Higher

TABLE 15 Turbidity and rainfall distribution in Leonard Springs Branch (LS) and Richland Creek (R) during the period 21 March-22 April 1964. (T = trace precipitation).

Turbidity (ppm SiO ₂) at stations:							
Date	R1X	R2	R3	R4	LS1	LS4	LS2
3/21/64	5	5	4	4	7	—	8
4/4/64	10	10	9	7	35	40	35
4/22/64	9	9	10	10	60	55	60

Rainfall (inches) by date:															
March								April							
	21	22	25	26	27	30	2	3	6	12	13	19	20	21	22
Inches	.05	.05	.23	.26	T	T	.49	.89	1.3	.01	.12	.11	.56	.06	.49

turbidity values for these seasons in Richland Creek probably result from the activity of man and cattle in the immediate vicinity of Richland Creek during summer and autumn. Comparable activities do not occur in Leonard Springs Branch.

The exact role of turbidity in the ecology of these planarians is not readily apparent. However, high turbidity at LS1 may partly account for the covering of slit and CaCO₃ on stones at this station, which in turn seems related to egg capsule deposition in *P. g. gracilis*, a point more fully discussed later in connection with substrate.

Substrate and changes in bottom character: The nature of the substrate referred to earlier in a general way (Table 1) requires some elaboration in order to more fully define the environment of the triclads. No quantitative measurements of bottom stability are available, but qualitative observations suggest some important differences between stations.

The flat, angular rocks of Station LS1 provide a substrate not easily moved by the swift current. This same type of substrate occurs all the way down to LS4, being gradually replaced by more rounded stones of rubble and gravel size. Some shifting of the stream bottom occurs at LS4, but the change in bottom character appears to be very slight. The type of substrate at LS1 and LS4 supports large numbers of planarians. However, at LS2, where planarians are scarce, the stream bottom undergoes noticeable change throughout the year, with bars of sand and gravel often forming in the stream. Once the max-min thermometer at LS2 was completely buried by a sand bar in two weeks.

Boulders and large pieces of angular rubble comprise most of the substrate at R2, and the bottom is relatively stable throughout most of the year. Hence this station, as LS1 and LS4, has a substrate inhabited by many planarians. An outstanding feature of the stream bottom in the vicinity of R3 is the pavement-like bedrock with few loose rocks and crevices. Such a substrate affords a poor refuge for planarians, and

any sudden increase of current velocity would sweep them downstream. Some rubble and gravel occur at R3 but continually are shifting. At R3 a max-min thermometer was buried by a gravel bar during a period of high discharge. The stream bottom seems more stable at R4 than at R3, and a large pool containing rubble and gravel harbored many *D. tigrina*.

Evaluation of observations on chemical features and other physical features: In general, planarians at all stations are subjected to rather extensive variations of calcium, magnesium, chloride, turbidity, specific conductance, and water level. As suggested, turbidity may play a role in the distribution of *P. g. gracilis*, but there is apparently no distributional relationship for chloride, magnesium, or specific conductance. Field observations coupled with general knowledge of triclade ecology suggest additional relationships for calcium, dissolved organic matter, water level (discharge), and substrate and changes in bottom character. These relationships are amplified in later discussion.

LABORATORY OBSERVATIONS

Temperature acclimation in the triclads

Field observations indicate that temperature is playing an important role in the distribution and abundance of the four species. These laboratory observations were made in order to understand more clearly the nature of this relationship.

Acclimation to gradual changes of temperature. An experiment was conducted to determine the capacity of each species to acclimate to bi-weekly temperature changes of 2°C over a range of 1°-35°C. The lethal temperature was that at which 50% mortality occurred during a two-week period. In addition, the ability of planarians to right themselves when inverted (righting reaction) was used as an index to the activity of each species during acclimation, and the reproductive capacity of each species at different temperatures was observed.

Sixty vigorous and relatively large individuals of each species were divided into three groups designated A, B, and C. Each group of 20 planarians was placed in a white, enameled pan containing approximately 500 ml of charcoal-filtered water. All triclads were maintained at 13°C, the starting temperature, for 6 weeks prior to the experiment. Group A of each species was then subjected at biweekly intervals to temperature increments of $2 \pm 0.2^\circ\text{C}$ over a range of 15-35°C or until lethal temperature developed. Groups B were subjected at biweekly intervals to temperature decrements of $2 \pm 0.2^\circ\text{C}$ over a 11-1°C range. Group C was maintained at $13 \pm 0.2^\circ\text{C}$ throughout the experiment.

For all temperatures from 1 to 31°C inclusive, the triclads were kept in thermostatically controlled rooms for which continuous records of air temperature are available. A thermostatically controlled incubator was used for temperatures greater than 31°C. In all instances water temperature was measured daily. Each pan of planarians was covered with a glass plate and kept in the dark throughout the experiment except during times of observation and feeding. The water was aerated vigorous-

ly at least once per day and more often at higher temperatures. All flatworms were fed weekly throughout the experiment on fresh beef liver, and the water was changed after each feeding. Water used for each change was always at the same temperature as that of acclimation.

The experiment is described under three headings: (1) acclimation capacity, (2) activity as reflected in righting reaction time, and (3) reproduction at different temperatures.

1. *Acclimation capacity.* All four species exhibited 100% survival over a range of 1-25°C. *P. g. gracilis* began to die after 3 to 4 days at 27°C and suffered greater than 50% mortality after 10 days at 27°C. Disintegration commenced in *C. foremani* after 10 days at 31°C, and only 15% survived after 11 days at that temperature. Ten percent of *D. dorotocephala* died after two weeks at 31°C, and 50% mortality occurred after 4 days at 33°C. *D. tigrina* showed 100% survival at 33°C for two weeks and 65% survival after two weeks at 35°C, at which time the experiment was terminated because of malfunction of the incubator.

Thus *D. tigrina* has the greatest acclimation capacity and *P. g. gracilis* the least capacity. *C. foremani* and *D. dorotocephala* are somewhat intermediate, with the former having less acclimation capacity than the latter.

2. *Righting reaction time.* The response of a planarian when placed on its back is to right itself. The time required to complete this righting reaction depends largely on the animal's physiological state, which in turn depends partly on the water temperature (Pearl, 1902; Bläsing, 1953). A planarian that is affected adversely by some external environmental factor such as temperature rights itself more slowly.

Each triclad in groups A, B, and C of all species was turned over gently with a stainless steel spatula, and the time for completing the righting reaction was measured with a stopwatch to the nearest 0.1 sec. The mean time was then determined for each group. This procedure was employed initially at 13°C for each species and once biweekly thereafter as follows: group A was measured at each temperature increment from 15°C to either 31°C or its lethal temperature below 31°C, group B was measured at each temperature decrement from 11° to 1°C, and group C was measured at 13°C for 18 weeks.

For each species the mean time required for each group to complete the righting reaction at the different temperatures is shown in Figure 22. Hereafter, the mean time required to complete the righting reaction will be referred to as "righting time." Statistical data for the experiment appear in Table 16.

Righting time is influenced not only by temperature. Some of the variation in this reaction may have resulted from changes in length of the triclads during the experiment, *i.e.*, longer triclads apparently require more time to complete the righting reaction (Pearl, 1902). Although detailed data on righting time as a function of length regrettably were not obtained, observations on changes in average length of each group of worms (Table 17) aid in the interpretation of Figure 22.

Since all three species for which data are available experienced a substantial decrease in average length over the temperature range of 13-31°C, one can assume that *P. g. gracilis* also decreased in length over

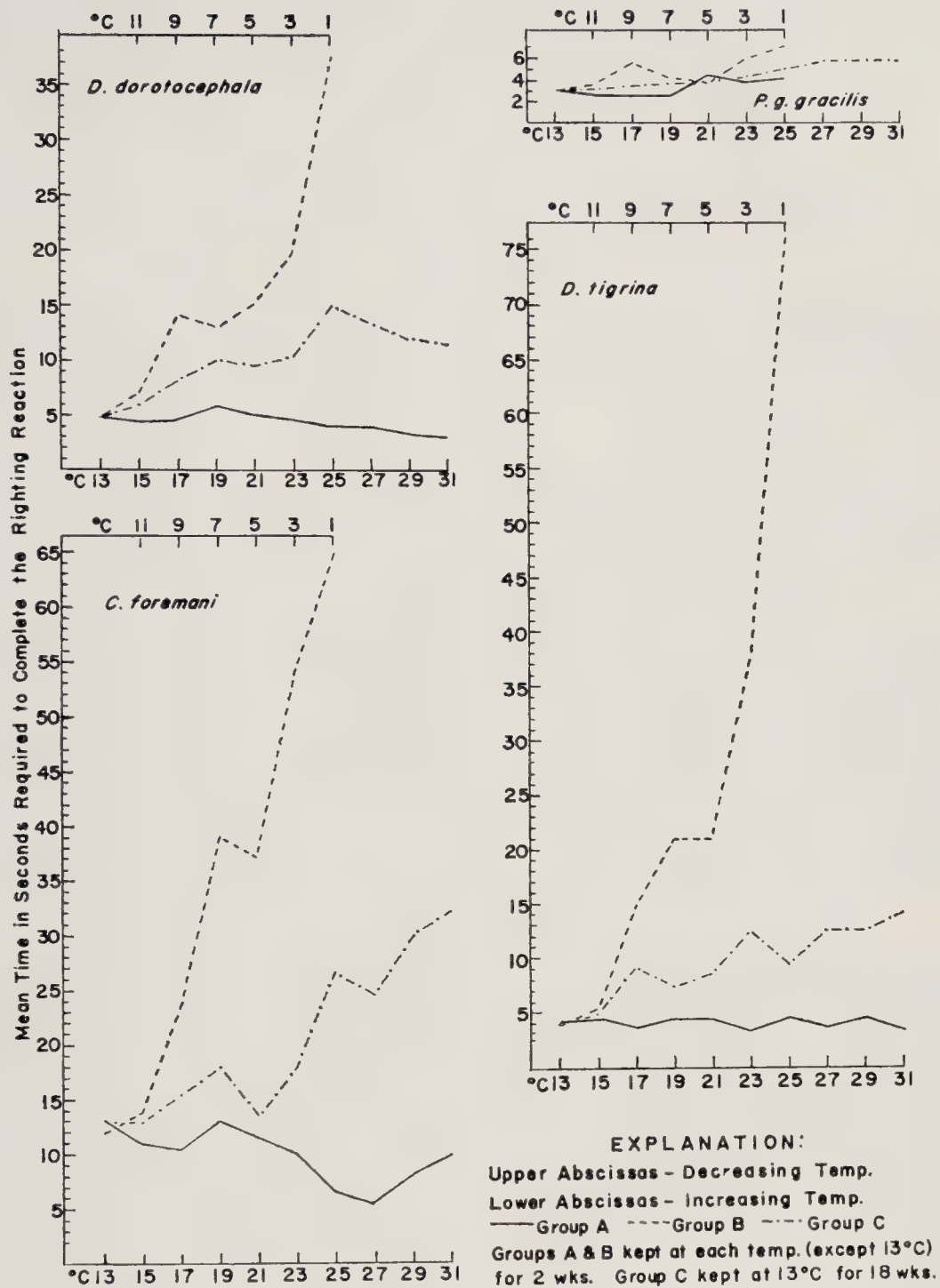


FIG. 22. Mean time (sec.) required for the four species of triclads to complete the righting reaction at different temperatures. Each point on the curves for groups A and B represents a single biweekly measurement made at the corresponding acclimation temperature. Each point on the curves for groups C represents a single biweekly measurement made at 13°C. Acclimation temperatures for groups A are on the lower abscissa, and acclimation temperatures for groups B are on the upper abscissa.

TABLE 16 Mean righting time (sec.) \pm standard error (s.e.) and number of triclads measured (n) for each group of all species at different temperatures ($^{\circ}\text{C} \pm 0.2$) (T).

Date	Group A		Group B		Group C	
	Righting time		Righting time		Righting time	
	T	$\bar{x} \pm \text{s.e.}(n)$	T	$\bar{x} \pm \text{s.e.}(n)$	T	$\bar{x} \pm \text{s.e.}(n)$
<i>C. foremani</i>						
9-24-64	13	$13.1 \pm 1.18(20)$	13	$12.2 \pm 1.39(20)$	13	$13.3 \pm 1.32(20)$
10- 8-64	15	$10.8 \pm 0.83(20)$	11	$13.6 \pm 1.21(20)$	13	$12.9 \pm 1.35(20)$
10-22-64	17	$9.5 \pm 0.81(20)$	9	$24.3 \pm 1.75(20)$	13	$15.5 \pm 1.13(20)$
11- 5-64	19	$12.9 \pm 1.36(20)$	7	$38.8 \pm 4.41(20)$	13	$18.0 \pm 2.23(20)$
11-19-64	21	$11.4 \pm 1.23(20)$	5	$36.9 \pm 3.11(20)$	13	$13.4 \pm 1.90(20)$
12- 3-64	23	$10.2 \pm 1.22(20)$	3	$54.1 \pm 5.32(20)$	13	$17.9 \pm 1.53(20)$
12-17-64	25	$6.6 \pm 0.87(20)$	1	$65.2 \pm 6.94(19)$	13	$26.5 \pm 2.26(20)$
12-31-64	27	$5.6 \pm 0.33(19)$			13	$24.6 \pm 2.03(20)$
1-14-65	29	$7.7 \pm 1.48(19)$			13	$30.3 \pm 3.04(19)$
1-28-65	31	50% mortality			13	$31.9 \pm 3.10(19)$
<i>D. dorotocephala</i>						
9-24-64	13	$5.3 \pm 0.23(20)$	13	$5.3 \pm 0.02(20)$	13	$5.0 \pm 0.02(20)$
10- 8-64	15	$4.6 \pm 0.02(20)$	11	$6.9 \pm 0.49(20)$	13	$6.1 \pm 0.38(20)$
10-22-64	17	$4.7 \pm 0.31(20)$	9	$14.1 \pm 1.55(20)$	13	$8.3 \pm 0.90(20)$
11- 5-64	19	$5.9 \pm 0.36(20)$	7	$13.0 \pm 1.19(20)$	13	$10.1 \pm 0.56(20)$
11-19-64	21	$5.2 \pm 0.34(20)$	5	$15.3 \pm 0.85(20)$	13	$9.6 \pm 0.62(20)$
12- 3-64	23	$4.8 \pm 0.33(20)$	3	$19.7 \pm 1.65(20)$	13	$10.3 \pm 0.57(20)$
12-17-64	25	$4.1 \pm 0.23(20)$	1	$37.5 \pm 3.51(20)$	13	$15.0 \pm 1.53(20)$
12-31-64	27	$4.0 \pm 0.02(20)$			13	$13.4 \pm 1.57(20)$
1-14-64	29	$3.3 \pm 0.01(20)$			13	$11.8 \pm 0.11(18)$
1-28-65	31	$2.9 \pm 0.01(20)$			13	$11.8 \pm 0.66(19)$
<i>D. tigrina</i>						
9-24-64	13	$4.4 \pm 0.02(20)$	13	$4.2 \pm 0.02(20)$	13	$4.0 \pm 0.01(20)$
10- 8-64	15	$4.7 \pm 0.29(20)$	11	$5.4 \pm 0.31(19)$	13	$5.2 \pm 0.29(20)$
10-22-64	17	$3.7 \pm 0.02(20)$	9	$15.1 \pm 1.54(19)$	13	$9.4 \pm 0.77(20)$
11- 5-64	19	$4.6 \pm 0.27(20)$	7	$20.8 \pm 1.89(17)$	13	$7.5 \pm 0.39(20)$
11-19-64	21	$4.4 \pm 0.32(20)$	5	$21.1 \pm 1.79(18)$	13	$8.7 \pm 0.64(20)$
12- 3-64	23	$3.4 \pm 0.66(18)$	3	$36.3 \pm 2.99(17)$	13	$12.5 \pm 1.00(20)$
12-17-64	25	$4.5 \pm 0.29(19)$	1	$75.6 \pm 5.76(17)$	13	$9.5 \pm 0.68(20)$
12-31-64	27	$3.8 \pm 0.02(19)$			13	$12.5 \pm 0.79(20)$
1-14-65	29	$4.5 \pm 0.23(20)$			13	$12.5 \pm 0.64(20)$
1-28-65	31	$3.5 \pm 0.02(20)$			13	$14.2 \pm 0.12(19)$
<i>P. g. gracilis</i>						
9-24-64	13	$3.3 \pm 0.02(20)$	13	$3.3 \pm 0.23(20)$	13	$3.2 \pm 0.02(20)$
10- 8-64	15	$2.7 \pm 0.01(20)$	11	$3.7 \pm 0.21(20)$	13	$2.9 \pm 0.02(20)$
10-22-64	17	$2.4 \pm 0.02(20)$	9	$5.5 \pm 0.71(20)$	13	$3.4 \pm 0.32(20)$
11- 5-64	19	$2.3 \pm 0.01(20)$	7	$4.0 \pm 0.34(20)$	13	$3.7 \pm 0.02(20)$
11-19-64	21	$4.4 \pm 0.97(16)$	5	$3.5 \pm 0.28(20)$	13	$3.8 \pm 0.24(20)$
12- 3-64	23	$3.7 \pm 0.23(19)$	3	$5.5 \pm 0.39(20)$	13	$4.0 \pm 0.02(20)$
12-17-64	25	$4.1 \pm 0.02(19)$	1	$7.0 \pm 0.34(19)$	13	$4.8 \pm 0.41(20)$
12-31-64	27	50% mortality			13	$5.5 \pm 0.42(18)$
1-14-65					13	$5.4 \pm 0.41(20)$
1-28-65					13	$5.4 \pm 0.33(19)$

TABLE 17. Changes in mean length (mm) of triclads during temperature experiments. Groups A subjected to 2° increases in temperature from 13 to 29°C over a 12-week period. Groups B subjected to 2° decreases in temperature from 13 to 1°C over a 6-week period. Groups C maintained at 13°C for 18 weeks. Initially there were 20 worms in each group for each species. No data are available for *P. g. gracilis* at 29°C because of mortality at lower temperatures.

	<i>C. foremani</i>				<i>D. dorotocephala</i>				<i>D. tigrina</i>				<i>P. g. gracilis</i>			
Group	13°	13°	29°	1°	13°	13°	29°	1°	13°	13°	29°	1°	13°	13°	29°	1°
A	11	————→	6		21	————→	15		12	————→	7		10	————→	?	
B	10	————→		10	17	————→		16	10	————→		9	10	————→		10
C	10	→	12		20	→	22		13	→	13		10	→	12	

its range of 13-25°C. *D. dorotocephala* and *C. foremani* exhibited a significant although irregular decrease in righting time over this temperature interval, whereas the other two species show no consistent trend. Hence, in these experiments shorter animals were not invariably associated with a quicker righting time.

All batches of worms subjected to decreasing temperatures (group B) showed essentially no change in average length over the 12-week period of the experiment (Table 17), although righting time in three of the species increased dramatically at the lower temperatures (Fig. 22). This is clearly a metabolic (temperature) effect, as reaction time in relation to length of worms is not a consideration. Of the four species, *P. g. gracilis* is the most active at lower temperatures as indicated by its comparatively rapid righting time (Fig. 22). *C. foremani* seems inherently more sluggish than the other species as evidenced by its comparatively greater righting time at 13°C (Fig. 22).

The control batches (group C) kept at 13°C for 18 weeks tended to increase in average length (Table 17), and all four species experienced an increase in righting time over this period, *P. g. gracilis* not quite so sharply as the other three species (Fig. 22). Again, the possible relationship between length of worms and righting time is not clear. *D. tigrina*, which experienced no increase in average length, had a significant increase in righting time. Evidently there is some long term acclimation at a given temperature, such as 13°. This is shown not only by the progressive changes in righting time and average length but also in the somewhat greater rates of reproduction than would be expected from the curves of the 2-week experimental series (see below).

3. *Reproduction at different temperatures.* All species except *P. g. gracilis* reproduced readily during the experiment. The cause of reproductive failure of *P. g. gracilis* is not definitely known, as all individuals seemed mature and quite active. Other laboratory observations described later suggest that the population density (20 triclads in each pan) was not high enough to stimulate reproduction in this species.

D. dorotocephala and *D. tigrina* reproduced only asexually and *C. foremani* only sexually. The reproduction accomplished by each species at different temperatures is shown in Figure 23. None of the three

species reproduced below 9°C. *D. dorotocephala* produced only 2 fission pieces below 13° but 153 over a range of 15-31°C, whereas the 20 individuals maintained at 13° produced 72 fission pieces during the 18-week period, or an average number of 8 per 2 weeks. *D. tigrina* produced 82 fission pieces over a range of 19-31°C, whereas the worms maintained at 13° produced only 4 fission pieces. *C. foremani* deposited 83 capsules over the range 17-27°C, which yielded 335 progeny. The worms maintained at 13° deposited only 3 capsules. However, other laboratory observations described later indicate that *C. foremani* is quite prolific at 13°C over a period longer than 18 weeks.

Apparently, increasing temperature stimulates reproduction in all those species shown in Figure 23. But, the temperature range over which reproduction occurs is rather wide for *D. dorotocephala* (13-31°C), somewhat narrower for *D. tigrina* (19-31°C), and narrowest for *C. foremani* (17-27°C). Temperatures below 19°C apparently inhibit reproduction in *D. tigrina*, whereas both the other species can reproduce well at 13°C. Considering optimum temperature for reproduction as that at which peak production occurs, this is 21°C for *C. foremani* and *D. dorotocephala* and 25°C for *D. tigrina*, based on two-week periods.

Acclimation to sudden increases of temperature. Different sets of planarians, which had been kept at 13°C for several weeks, were subjected to sudden increases of temperature by transfer to a thermostatically controlled room at a higher temperature. Twenty different individuals of each species were used in each transfer.

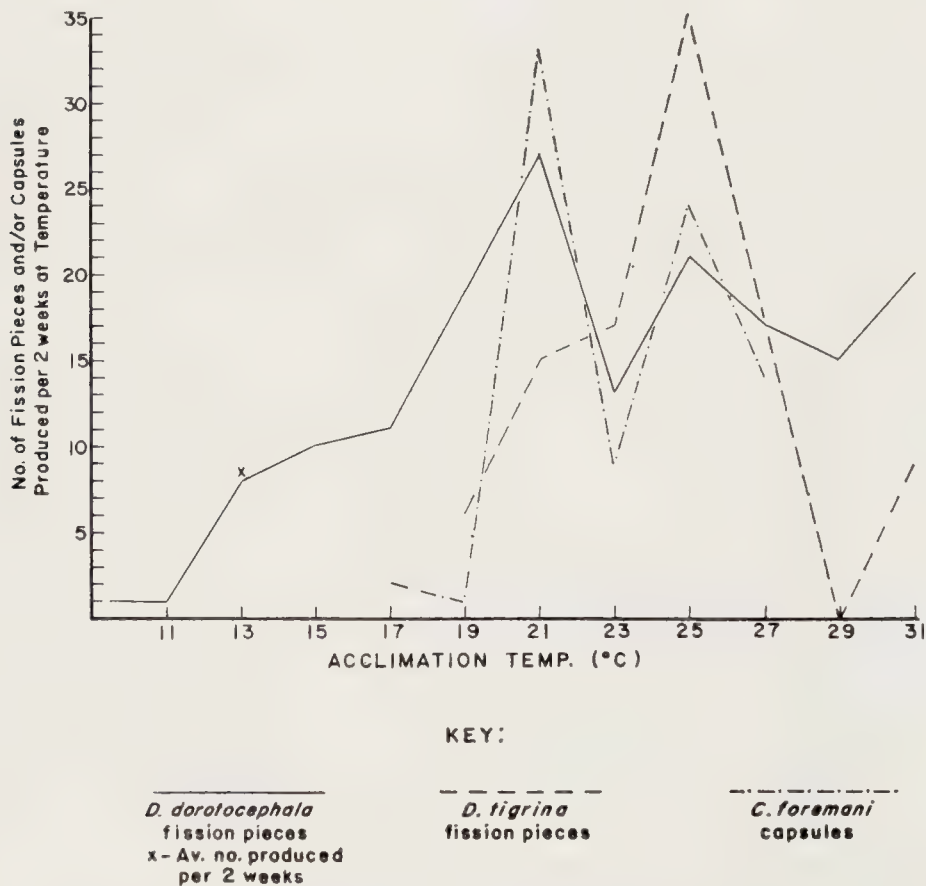


FIG. 23. Reproductive capabilities of *C. foremani*, *D. dorotocephala*, and *D. tigrina* at different acclimation temperatures.

1. *Transfer of C. foremani, D. dorotocephala, D. tigrina, and P. g. gracilis from 13 to 25°C.* The higher temperature chosen was 2°C below that at which *P. g. gracilis* of the earlier experiment had suffered 50% mortality. The water temperature of the pans reached 25°C in about 2.5 hrs after transfer. Although righting times were not measured, the higher temperature produced no immediately visible change in the behavior or appearance of triclads. One individual of *P. g. gracilis* disintegrated at the end of three days, and six others were in the process of disintegration. *P. g. gracilis* suffered 50% mortality after two weeks at 25°C. All individuals of the other three species survived the transfer with no apparent ill effects.

2. *Transfer of C. foremani, D. dorotocephala, and D. tigrina from 13 to 29°C.* The higher temperature chosen was 2°C below that at which *C. foremani* of the earlier experiment had incurred 50% mortality. The water temperature of the pans reached 29°C in about 3 hrs after transfer, and approximately 21 hrs later all individuals of *C. foremani* had disintegrated. *D. dorotocephala* and *D. tigrina* showed 100% survival after two weeks at 29°C. A repeat of this transfer, using another set of worms, gave the same results.

Apparently, *C. foremani* and *P. g. gracilis* are least able to acclimate to sudden and relatively large increases of temperature, whereas *D. dorotocephala* and *D. tigrina* seem little affected under similar circumstances.

Additional laboratory observations

Twenty individuals of each species were kept under observation for seven consecutive months at 13°C, being maintained as previously described. During this period, *C. foremani* deposited 82 capsules and produced 489 progeny, *D. dorotocephala* produced 126 fission pieces, and *D. tigrina* produced 12 fission pieces. No reproduction occurred in *P. g. gracilis*.

Field collections of *P. g. gracilis* were usually kept in separate pans containing 20 to 50 individuals, since these numbers facilitated feeding and water changes. These were maintained at 13°C as described earlier. No reproduction was observed in any of these pans for approximately 18 months. In February of 1965 the contents of several pans were combined into one, resulting in a single pan with about 200 individuals. In April of 1965, 50 egg capsules were found in this pan, suggesting that a sufficient population density may stimulate reproduction in *P. g. gracilis* under laboratory conditions. This may explain why *P. g. gracilis* failed to reproduce during the acclimation experiment, since only 20 individuals were in each pan.

Observations on feeding behavior of the four species were made using the amphipods *Crangonyx* and/or *Gammarus* as food. The amphipods were slightly immobilized by pinching them with forceps and then were placed in pans containing flatworms. All four triclad species fed upon the amphipods, thus substantiating field observations concerning feeding.

DISCUSSION

The environmental factors that seem directly related to the distribution and abundance of the four triclads species are temperature, water level (discharge), substrate and changes in bottom character, calcium, and dissolved organic matter. However, this is not to imply that the other factors investigated are not involved in the ecology of these planarians, only that no direct relationship was found.

Temperature

Of the physical and chemical parameters, temperature seems to be the most important in affecting distribution of the four species. Compared to the downstream stations, temperature is less variable at the upstream stations of both streams on a daily, seasonal, and annual basis. This is especially true at Station LS1. Correlated with this thermal stability, each triclad species except *D. tigrina* is found more abundantly upstream. Not only are average abundance indices greater at LS1 (4.3), LS4 (4.9), and R2 (3.6) than at LS2 (1.2) and R3 (1.5), but the relative density of planarians at each station shows a similar relationship.

D. tigrina does not appear in headwater collections and is the only abundant species of planarian at R4. This suggests that it is most successful in downstream areas where summer temperatures are higher, and warmer periods are of greater duration. At R4, *D. tigrina* is numerous in pools that occasionally reach 32°C in summer. Hyman (1939, 1951b) and Kenk (1935, 1944) report that it favors slowly-flowing or stagnant waters and is seldom found in springs. Of the four species in this study, *D. tigrina* has the greatest acclimation capacity to gradual increases of temperature, and it apparently shows no ill effects on being suddenly transferred from 13° to 29°C. Likewise, *D. tigrina* shows little activity at temperatures below 11°C. Comparing its reproductive capability at different temperatures, *D. tigrina* produces most fission pieces at 25°C and exhibits little or no asexual reproduction below 19°C. All these observations suggest that *D. tigrina* is prevented from colonizing cooler waters upstream at least in part because of inability to be active and reproduce well at lower temperatures.

Reynoldson *et al.* (1935) note that the genus *Phagocata* tends to be associated with a cold micro-habitat. The distribution of *P. g. gracilis* in my study indicates that of the four species it is most closely restricted to relatively cool waters that show little annual temperature fluctuation. It was collected regularly only at Stations LS1 and LS4, and when found in other streams of the area it was always associated with headwater situations similar to those of Leonard Springs Branch. The apparent preference of *P. g. gracilis* for cooler waters is further evidenced from a study by Eddy and Gleim (1932), who found that subjection of these triclads to a temperature gradient of 0-35°C for 30 min. resulted in congregation of the worms in a 0-9.5°C range.

Laboratory observations show that *P. g. gracilis* is the most coldly stenothermal of the four species. It has the lowest acclimation capacity to gradual increases of temperature and incurs 50% mortality when the

temperature is suddenly increased from 13° to 25°C. This suggests that it could scarcely tolerate the extreme summer temperature fluctuations sometimes occurring at Stations LS2, R2, R3, and R4. The comparatively rapid righting time of *P. g. gracilis* at lower temperatures indicates its ability to be active in cool waters. Its failure to reproduce during the acclimation experiment precludes any definite conclusions about optimum temperature for reproduction; however, its life cycle as inferred from population-size structure at LS1 suggests that most breeding occurs at lower temperatures during winter and early spring.

The greater abundance of *D. dorotocephala* at Station LS4 than at LS2 suggests that it is more successful in waters of rather constant temperature; however, it continually occupies areas of Leonard Springs Branch having extensive temperature fluctuation. Laboratory observations show that *D. dorotocephala* acclimates well to higher temperatures, whether they are gradually or suddenly imposed. Although 21°C seems to be optimum temperature for asexual reproduction, the species reproduces well over a wide temperature range. Compared to *D. tigrina*, its activity at lower temperatures is not drastically reduced. Although field observations seem partly contradictory, laboratory observations indicate that *D. dorotocephala* is eurythermal and that temperature is not influencing its distribution to the extent that it does for *P. g. gracilis* in Leonard Springs Branch.

C. foremani is more abundant in the headwaters of Richland Creek where extremes of temperature are not of the same magnitude and duration as those at Stations R3 and R4, but, like *D. dorotocephala*, it regularly appears in downstream areas. Thus, it seems from these observations that *C. foremani* is also eurythermal. However, laboratory observations indicate that temperature is partly responsible for the greater abundance of *C. foremani* upstream. Although it acclimates well to gradual increases of temperature, it cannot tolerate a sudden increase from 13° to 29°C. The species is more prolific at temperatures below 23°C, suggesting that it might be less reproductively successful in warmer downstream areas; indeed, no capsules of *C. foremani* were found at R3 or R4. Thus, temperature largely influences the distribution of *C. foremani* through its effect on reproduction.

This study corroborates similar studies in Europe that identified temperature as a major factor in the distribution of planarians (see Dahm, 1958, for discussion). It is possible that if the four triclad species were found abundantly in the same stream they would be linearly distributed: *P. g. gracilis* would occupy the cooler upstream areas; *C. foremani* and/or *D. dorotocephala*, the zone of intermediate temperatures; and, *D. tigrina*, the warmer waters downstream. Such a distribution actually occurs among three species of stream-dwelling triclads in Europe and Japan (Thienemann, 1925). Temperature is exerting an influence on distribution partially through its effect on the reproductive activity of at least three of the four species in this study, a relationship recently clarified for four other planarian species in England (Reynoldson *et al.*, 1965).

Water level

Variations in discharge as reflected by changes in water level apparently have considerable influence on triclad abundance (Fig. 24). All stations show a general inverse relationship between abundance and discharge. Comparison of Station LS1 with Station R2 shows that both were equally abundant in triclads during autumn of 1963, and both experienced similar increases of discharge in winter and spring. However, the decrease in triclad abundance during high water was much more pronounced at R2, thereby suggesting that *C. foremani* at R2 is less able to maintain position during periods of high discharge than is *P. g. gracilis* at LS1 and that the former would probably be unsuccessful in the continually faster current of LS1.

Substrate and changes in bottom character

As many other stream invertebrates, planarians are more abundant at stations where the substrate is not easily moved by the current, and in this respect, flattened, angular stones seem to afford a better substrate than more rounded rocks of gravel size. The low number of triclads at Stations R3 and LS2 resulted partly from pronounced changes in the character of the stream bottom. Any extensive movement of the bottom results in molar action that causes injury and death to soft-bodied animals such as planarians.

The distribution of *P. g. gracilis* may be influenced by a particular type of substrate, *viz.*, stones having an encrustation of silt and CaCO_3 . These occur abundantly in the upper reaches of Leonard Springs Branch but not downstream nor in Richland Creek. Capsules of *P. g. gracilis* being unstalked obviously require some other means of avoiding being swept downstream by the swift current in the upper reaches of Leonard Springs Branch. The worms in some way deposit these capsules under the encrustation, thus protecting them effectively from the current, indeed a unique adaptation in running water.

Chemical features

In discussing the relationship between calcium and the distribution of freshwater organisms, Macan (1963) comments that certain groups seem to be favored by calcium. Presumably all four species in this study fall into this category, since calcium levels are always relatively high in both streams. Such conditions provide a favorable environment for planarians in terms of chemical stability and generally high productivity.

Hyman (1951a) notes that *D. dorotocephala* is probably limited to waters of high calcium content. However, the exact way in which calcium exerts a limiting effect is apparently unknown.

Planarians cannot tolerate high degrees of pollution over a long period, and they have sometimes been used as indicator species in pollutional studies (Kolkwitz, 1950; Patrick, 1950). However, enrichment of water through mild pollution may stimulate an increase in numbers of some triclad species (Macan, 1962). Results of the present study suggest

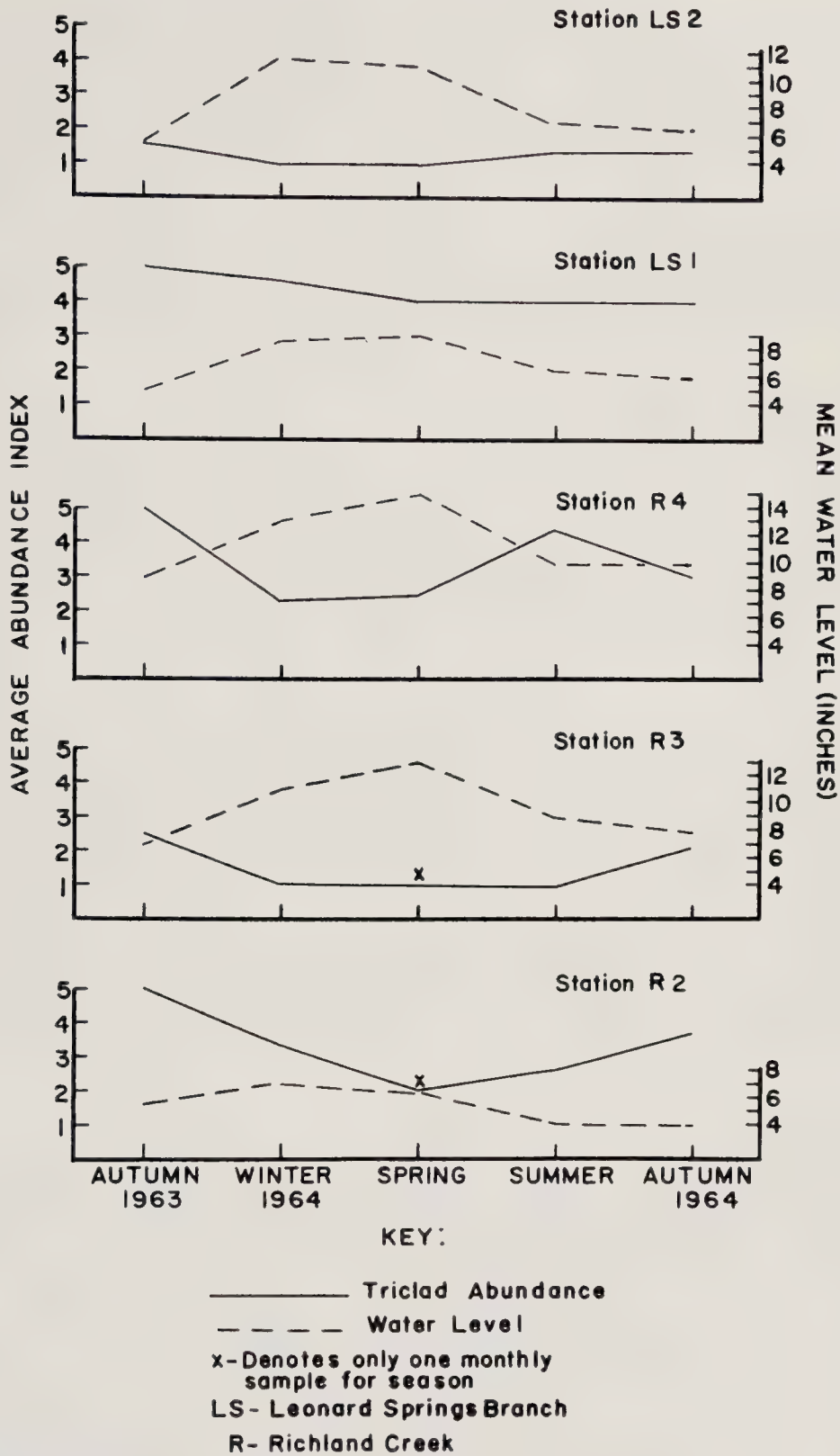


FIG. 24. Average abundance index of triclads and mean water level (inches) for each season at stations on Leonard Springs Branch and Richland Creek.

that pollution is not so extensive as to be deleterious to the triclad fauna of either stream but is important in increasing levels of stream production, which in turn promote large numbers of planarians.

There is the possibility that somewhat higher levels of dissolved organic matter in Richland Creek may exclude *D. dorotocephala*, since it is reputedly very sensitive to putrefactive material in water (Hyman, 1925).

Triclad s at each station are generally subjected to relatively wide variations of the other features investigated, implying they are euryecious as regards these features in Leonard Springs Branch and Richland Creek.

Food relationships

Amphipods and isopods are a potential food source for triclad s, and their relative abundance in the two streams seems to coincide with that of planarians. cursory observations indicate that the scarcity of triclad s at LS2 and R3 seems related to the paucity of amphipods and isopods at these stations, whereas stations that have plenty of these crustaceans support a large number of triclad s. However, both may be covarying with some external factors rather than being dependent on each other.

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The Ecology of Invertebrates in an Intermittent Stream¹

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ABSTRACT

This study is concerned with the ecology and certain physiological mechanisms of macroinvertebrates in a small stream of south-central Indiana that becomes completely dry at the surface each summer. The stream fauna is dominated by two aquatic peracarid crustaceans, *Lirceus fontinalis* and *Crangonyx forbesi*, which occur in substantial numbers throughout the year. They survive the dry season as small animals in subsurface seepage and water-saturated air spaces of moist soil. The other aquatic animals of the stream can be divided into two groups in respect to season: a late summer-autumn association, characteristic of the stream when it is dry or nearly so, and a late winter-spring association characteristic of the stream when it is flowing or nearly so. The active summer-autumn group consists mainly of adventitious species and of adult aquatic beetles, which are permanent members of the stream. The winter-spring invertebrates are mainly permanent residents, which appear each year and constitute a uniform and stable community. With the possible exception of certain caddisflies, these species are not specifically adapted to the intermittent environment but in a sense are preadapted to it by certain favorable features of their life cycles.

L. fontinalis and *C. forbesi* have an annual cycle in the intermittent stream. Both begin to breed in March and April, months that normally exhibit the maximum amount of water in the stream. In *L. fontinalis* reproduction is terminated in summer by the early death of animals of the old generation that are still reproducing, and by an arrested growth of the new generation. Both phenomena are influenced by the onset of the dry period. In contrast, for *C. forbesi* reproduction is unvaryingly confined to the spring, with the females producing one brood and then dying prior to the drying up of the stream. Individuals of the new generations of both species grow very little during the dry summer and autumn months. Because of their small size they can survive in subsurface seepage and moist interstitial spaces. In late autumn and winter rapid growth resumes.

For *L. fontinalis* oxygen consumption declined in late spring and summer when measured at 5°C but not when measured at 13°C. If size is considered, however, the animals at 13°C also would exhibit a lower metabolic rate in summer. When exposed to water-saturated air in the laboratory, both *L. fontinalis* and *C. forbesi* from the intermittent stream lived for a longer period of time than did related organisms from a permanent stream.

Physical features of the stream bed, especially during the dry season, are pointed out as being of prime importance in determining the permanency of an aquatic fauna in small summer-dry streams. The availability of interstitial spaces is of special importance in this respect. Besides offering a suitable habitat for aquatic animals during the dry season, they are of importance when the stream is flowing by reducing the velocity of the water, which might otherwise flush out much of the aquatic fauna. The maintenance of interstitial spaces depends on local geological and geographical features. The presence of non-angular bottom materials, the absence of a heavy silt load, and protection from daily freezing and thawing during the winter are important in this respect.

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TABLE OF CONTENTS

	Page
Introduction	58
Description of study area	59
Methods	63
Seasonal distribution	65
Reproductive cycles of <i>Lirceus fontinalis</i> and <i>Crangonyx forbesi</i>	73
<i>Lirceus fontinalis</i>	73
<i>Crangonyx forbesi</i>	77
Generalized life cycles	80
Seasonal oxygen consumption, <i>L. fontinalis</i>	80
Humidity and soil factors	83
Humidity and survival of <i>L. fontinalis</i>	83
Soil samples	86
Discussion	90
Acknowledgments	95
References	96

INTRODUCTION

Many regions of the United States and elsewhere are characterized by very small streams, which usually flow for only a short period each year and regularly go completely dry in late summer and autumn. Such streams can make up a considerable part of the watershed system. These streams are numerous especially in south-central North America where large watersheds may exhibit only a few permanent or summer-interrupted streams, the remaining being the small intermittent or ephemeral type. One has only to examine a topographic map of a typical intermittent stream locality, such as parts of Kentucky, Indiana, and southern Illinois, to appreciate at least the areal importance of these small streams. The total kilometers of summer-dry streams may well exceed the total kilometers of permanent streams. Permanent streams, however, have received the major attention of aquatic biologists. This largely has been due to their greater abundance of aquatic life, especially fish, and to their role in waste disposal.

While the range of limnological research possibilities of intermittent and ephemeral streams would appear to be limited by their scanty fauna, the biological significance of such streams is much greater than would be expected on casual observation. In summer and early autumn they suggest either an environment completely devoid of aquatic life or certainly one that would present severe ecological and physiological stresses to any aquatic invertebrates living therein. After heavy rains of autumn, however, pools are formed which may at once be occupied by substantial numbers of aquatic invertebrates. In south-central Indiana the aquatic isopod *Lirceus fontinalis* Rafinesque and the aquatic amphipod *Crangonyx forbesi* (Hubricht and Mackin) especially are noteworthy in this respect. In spring when the streams are flowing, the faunae present resemble rather closely those of permanent streams of the region.

Such streams scarcely conform to our concept of a lotic environment in the same way as permanent streams. In fact over a year's time the streams could serve as common meeting grounds for terrestrial ecology, pond limnology, and stream limnology. Aquatic animals surviving in the streams must be subjected to severe and diverse environmental stresses—a flowing stream in spring, a pond situation in early summer, an apparent terrestrial situation in late summer and early autumn, and pools in late autumn and winter, which because of decomposing leaf material have a high oxygen demand and superficially resemble the hypolimnion of deep lakes.

The continuing presence of aquatic fauna despite regularly occurring dry periods prompted the investigator to question how these populations are maintained or how repopulation is accomplished. The migration of aquatic organisms into the stream via reestablished connections with permanent water bodies or via flight, as in adult insects, was a possibility and would be expected in many instances. There was also the possibility that a permanent aquatic fauna existed in such streams and that it might be typical and even restricted to such streams. How would the aquatic organisms survive the dry periods? Are there special adaptations or modifications in their life cycles that permit true aquatic animals to survive in apparently terrestrial localities? What is the nature of the dry stream bed, and is there a semblance of the aquatic environment below its surface, providing conditions amicable to life for such aquatic forms adapted to it? What, if any, contribution does the small intermittent and ephemeral stream fauna make to the productivity of associated permanent waters? These are some of the matters to be considered in determining the ecology and the biological importance of these very numerous intermittent and ephemeral streams.

This study is an initial attempt to elucidate the above problems by investigating the complex of life in a small stream that regularly goes completely dry in the summer and early autumn. The study included the seasonal community structure of the invertebrates and especially the ecology of the major invertebrates. In this respect reproductive cycles, metabolic rates, and soil and humidity factors were major points. Finally an attempt was made to develop faunistic parameters useful in classifying or at least arranging streams in a continuum with respect to duration of intermittency. Various aspects of the investigation were conducted from October 1963 to June 1965.

DESCRIPTION OF STUDY AREA

The stream chosen for study is known from topographic maps as Caldwell Hollow (Fig. 1). It is a tributary of Salt Creek, which in turn contributes to the West Branch of the White River system. The stream is located in the Norman Upland of Brown County, approximately 23 km east of Bloomington, Indiana. This is a hilly region, the entire area being dissected by small intermittent and ephemeral streams. Bedrock of the Upland is the Borden Formation characterized by sandstones, shales, and clays. Although glacial outwash material is present locally in major valleys, none is present in Caldwell Hollow. Springs are generally

absent in this region. The watershed of Caldwell Hollow is completely wooded, characteristic vegetation being elm (*Ulmus americana* L.), hickory (*Carya* sp.), black cherry (*Prunus serotina* Ehrh.), and beech (*Fagus grandifolia* Ehrh.). Sycamores (*Plantanus occidentalis* L.) are common at the lower end. The materials of the stream bed are predominately sandstone particles of various sizes and shapes. Bedrock exposures of sandstone and clays are present but infrequent.

Table 1 summarizes important morphometric and meteorologic features. Caldwell Hollow was continuous with Salt Creek for only 46 days during the period October 1963 to October 1964. For most of the study period the stream was a series of widely scattered shallow pools. The most prolonged dry spell occurred from August to November 1964. During this period the stream was completely dry for 38 days, and although water was present in September, it was restricted to one pool. Although the stream is short and has a rather steep gradient, torrential floods are

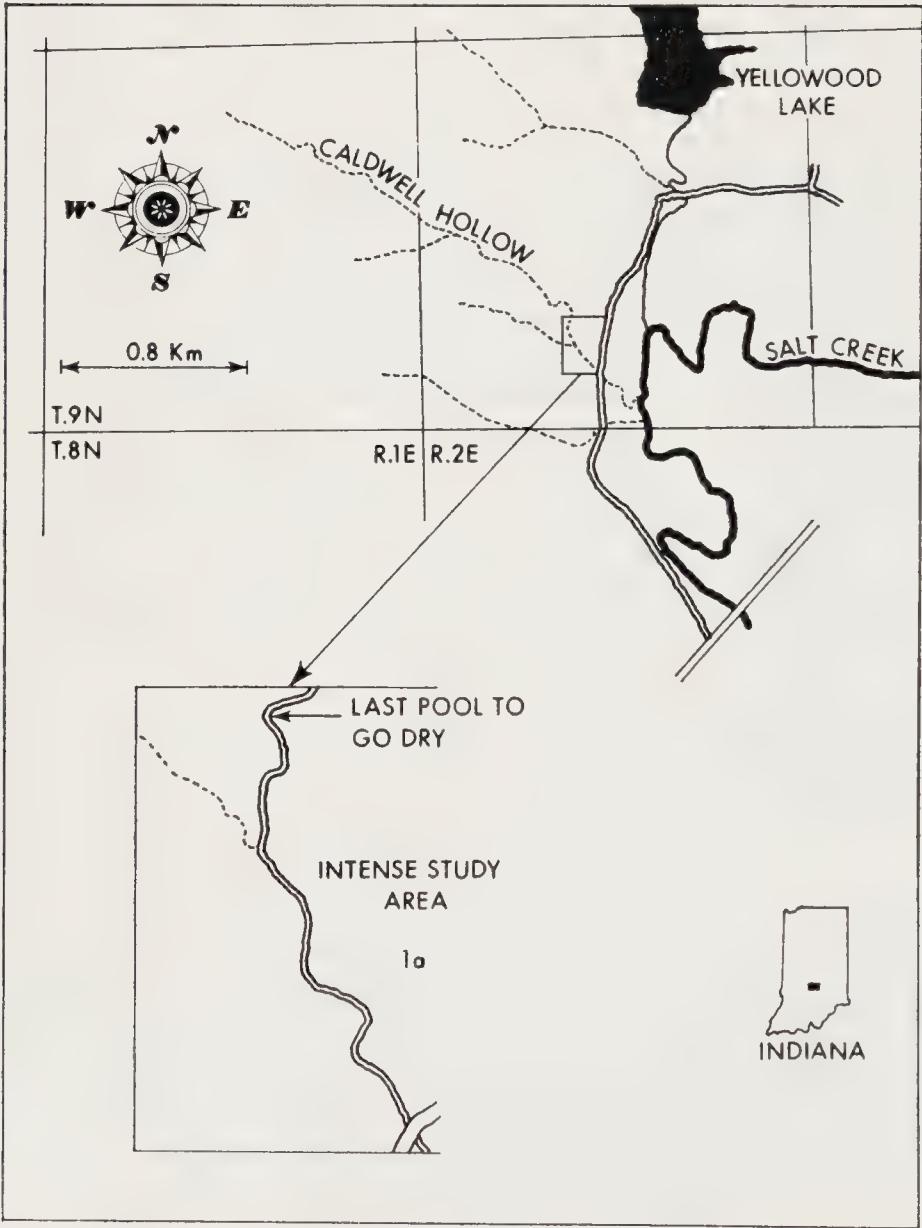


FIG. 1. Map of the study area.

rare. They probably are minimized by the large amount of lateral sub-surface drainage that can take place through the loosely packed layers of gravel-size sandstone. During the study period only one flood occurred. This was 8 March 1964, when the region received over two inches of precipitation. It completely altered large areas of the stream bed, scouring out new pools and filling in old ones with sandstone particles.

Water temperatures, dissolved oxygen, and the water conditions are portrayed in Figure 2. The stream was continuous from headwaters to mouth during most of March and April of 1964. In late spring and early summer it was reduced to isolated pools. In late summer and early autumn the stream was completely dry or exhibited only transitory pools after heavy rains. Pools that did form at this time were characteristically dark brown in color due to decomposition of leaf material. Water temperatures were high, and dissolved oxygen was near depletion. Larimore *et al.* (1959), Schneller (1955), and Slack (1955) investigated various aspects of the "black water" phenomenon, but much remains to be learned, especially concerning the possibilities of released toxins.

TABLE 1. Summary of morphometric and meteorologic data, Caldwell Hollow, Indiana—October 1963-March 1965.

Origin above sea level: 228 m					Area of watershed: 3.1 km ²	
Total flow length: 2.22 km					Av. gradient: 31.7m/km	
Flow length, study area: 0.32 km						
	No. days continuous with Salt Creek	No. days some flow in study area	No. days of isolated pools only	No. days completely dry	Monthly* precipitation (cm)	
					Total	24 hr. maximum
1963						
Oct.	0	0	19	12	0.38	
Nov.	0	0	31	0	5.38	2.67
Dec.	0	0	31	0	3.73	0.79
1964						
Jan.	0	11	20	0	5.49	1.35
Feb.	0	6	23	0	2.77	1.27
Mar.	27	4	0	0	20.70	5.69
Apr.	18	11	1	0	13.00	3.20
May	0	0	31	0	3.62	0.51
June	1	2	27	0	11.10	3.23
July	0	0	31	0	13.13	4.57
Aug.	0	0	14	17	3.28	2.21
Sept.	0	0	30	0	2.23	1.75
Oct.	0	0	22	9	0.89	0.46
Nov.	0	0	18	12	5.46	2.39
Dec.	0	6	25	0		
1965						
Jan.	12	14	5	0		
Feb.	22	6	0	0		
Mar.	31	0	0	0		

* Sources: U.S. Weather Station, Bloomington, Indiana

With the prolonged rains of autumn more permanent-type pools formed, and by January 1965 the stream was flowing again. Such initial flow usually resulted in the flushing out of accumulated leaf material and consequently in the flushing out of substantial numbers of aquatic isopods and amphipods. Although numerous sampling trips and observations were made from headwater to mouth, most of the intense work was conducted in the lower section comprising a study area of approximately 0.32 km (Fig. 1a). The last pool remaining before the stream went completely dry was located in this area.

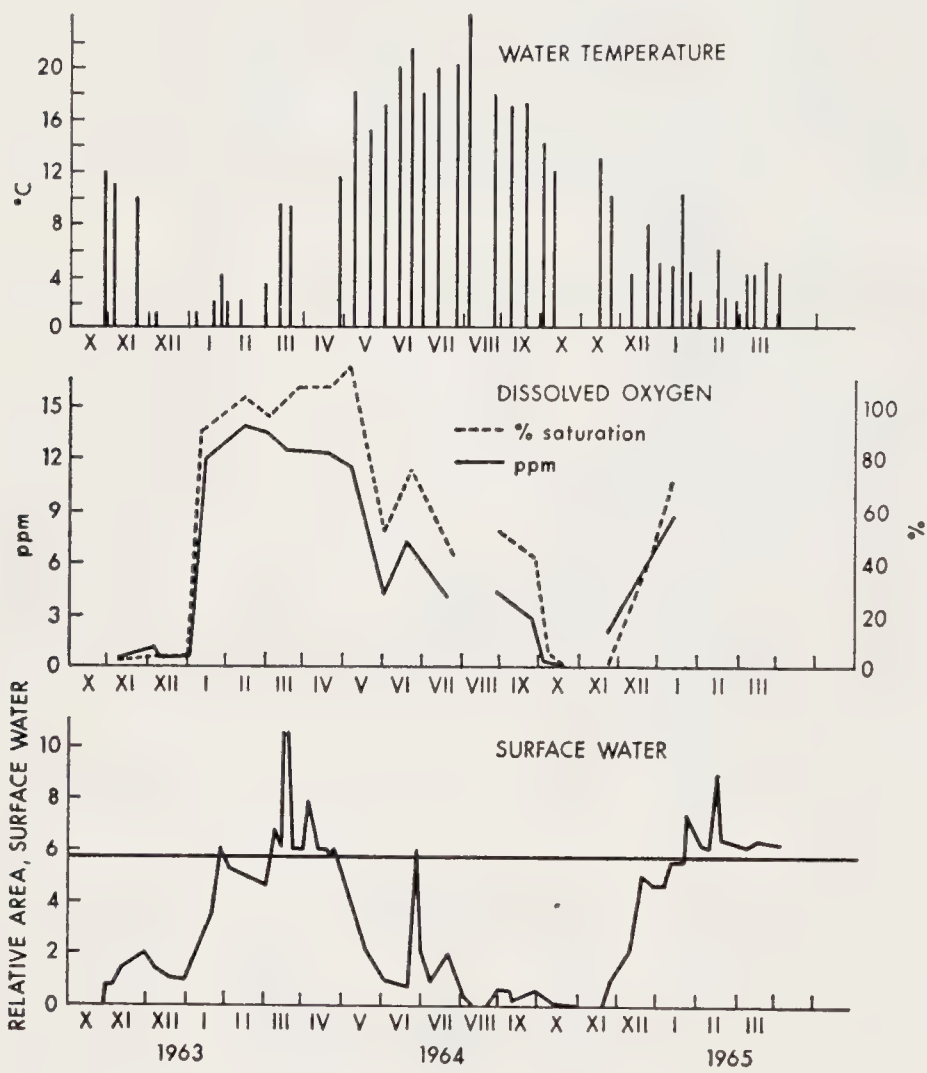


FIG. 2. Water temperature, dissolved oxygen, and visible surface water for study period, October 1963-March 1965. Surface water was determined by mapping the width and length of the stream bed on square-millimeter graph paper. Each time the stream was visited, the visible water was sketched on these maps and its total area in square millimeters calculated. When the relative area was above 5.8 (580 mm²), the stream was flowing from headwater to mouth; below 5.8 it was a series of isolated pools or completely dry.

METHODS

Spot qualitative sampling was performed each time the stream was visited, usually at weekly intervals (Table 2). The organisms were collected from as large an area of the stream as time would allow. On some occasions the stream was sampled from headwater to mouth. Samples were collected by various hand nets, shovels, and by picking organisms off rocks and leaves with forceps. When the stream was dry, soil samples were taken from former riffle and pool areas. These were brought into the laboratory and either placed in flowing water systems for the recovery of living organisms or spread out in a white enamel pan and slowly flooded with formalin. Living organisms were observed by this method. Emergence or migration of insects were periodically monitored by insect traps.

Quantitative samples were collected usually at slightly shorter than monthly intervals (Table 2). These samples were used for the reproductive cycles of *Lirceus* and *Crangonyx* and to compare numerically the composition of the fauna on a particular sampling date. Samples were collected with a nylon hand net having 12 threads per centimeter. This size net does not sample adequately very small organisms in the one- and two-millimeter size groups. Sampling was as randomly performed as possible by working the net through selected pools and riffles (when present) over the entire study area. Samples were hand sorted in the field, the animals being transferred to 70% alcohol until they could be identified and measured to the nearest millimeter. During the times it was necessary to collect animals when the stream was dry, large quantities of moist soil were collected, flooded with formalin, and the organisms collected by a sugar flotation technique (Anderson, 1959).

For investigating the reproductive cycles of *Lirceus fontinalis* and *Crangonyx forbesi*, each specimen was sexed and then measured to the nearest millimeter from the anterior tip of the cephalothorax to the posterior end of the telson. The modification of the first two pair of pleopods for use in copulation is diagnostic of male isopods, while the short curved, outward projecting outer ramus of uropod 2 is diagnostic of *Crangonyx forbesi* males. Mature males of this species have calceoli on antenna 2. Each specimen was then tabulated in one of several categories: males, females without oostegites or having nonbristled oostegites, ovigerous females, females with young, females with empty brood pouch, and juveniles. Juveniles were animals 6 mm in length or shorter for *Lirceus* and 5 mm in length or shorter for *Crangonyx*. The number of eggs or young of each female for both species was recorded. To investigate the number of broods and incubation time of each species, several pairs of one male and one female each were observed in the laboratory at 13° C. The animals were maintained on elm leaves, the diet of *Crangonyx* being supplemented periodically with proteinaceous food, usually freshly killed specimens of the same species.

Oxygen-consumption determinations were made at approximately monthly intervals. *L. fontinalis* was collected the day before the tests and held in a 5° C room without food. Tests were run at 5° C the following morning, the animals then immediately transferred to 13° C, and the test repeated at the latter temperature the next morning. By this procedure

the organisms were exposed to each test temperature for approximately 16 hours prior to measurements. Grainger (1956), working with crustaceans, found the initial overshoot in oxygen consumption to last for only a matter of minutes or an hour at most before reaching a steady level when the animals are transferred to a different temperature.

A 10-cc syringe was used as the respiratory chamber. This was filled by removing its plunger and submerging the barrel in air-saturated water. Four to eight test animals were then placed in the barrel, the barrel resubmerged, the plunger inserted to the 10-cc mark and the syringe tip sealed with an air-tight rubber cap. Initial oxygen determination of air-saturated water was made according to a micro-Winkler technique described by Burke (1962). At the end of two hours at 5° C or one hour at 13° C, the respiratory chamber was removed from its water bath and vigorously shaken. A hypodermic needle attached to a 5-cc syringe was inserted through the rubber cap of the respiratory chamber and a 5-cc sample drawn off by pressing down on the plunger of the respiratory chamber. This sample was used for the final micro-Winkler titration and estimation of oxygen consumption. After the 13° C test the animals were blotted on filter paper and wet weighed.

SEASONAL DISTRIBUTION

The active fauna can be divided into two groups in respect to seasons (Fig. 3): a late summer-early autumn association characteristic of the stream when it is dry or nearly so, and a late winter-spring association typical of the stream when it is flowing or nearly so. The two dominant crustaceans, *Lirceus fontinalis* and *Crangonyx forbesi* are found in substantial numbers throughout the year, surviving the dry periods as very small animals with little growth in deep subsurface seepage and moist interstitial spaces. Certain aspects of their ecology and physiology will be detailed in a later section.

Are the other animals permanent residents of the stream or are they migratory species? If they are permanently associated with the intermittent stream how do they survive the dry periods? If they are migratory species can they complete their life cycle in this environment? To cast light on these questions a certain amount of descriptive discourse is necessary. Paucity in numbers of the various species, with two or three exceptions, made the construction of growth histograms impractical. Another factor entering here was the very short but rapid growth period, especially of the hemimetabolous spring fauna. Large quantitative samples, collected at monthly intervals and not adequately sampling very small organisms, represented in some instances only a single phase in the species' growth, since by the next sampling period the species had emerged. Therefore spot sampling, although not satisfactory for size histograms, was relied on heavily for determining the animal's history.

Starting with the summer-autumn association, the adult aquatic beetles—*Limnebius discolor* Casey, *Cymbiodyta* sp., and *Hydroporus laetus* Leech—are permanent components of the stream. They survive the dry spells by actively burrowing deep into moist interstitial spaces of the stream bed. Larvae of *H. laetus*, the only abundant beetle in the stream,

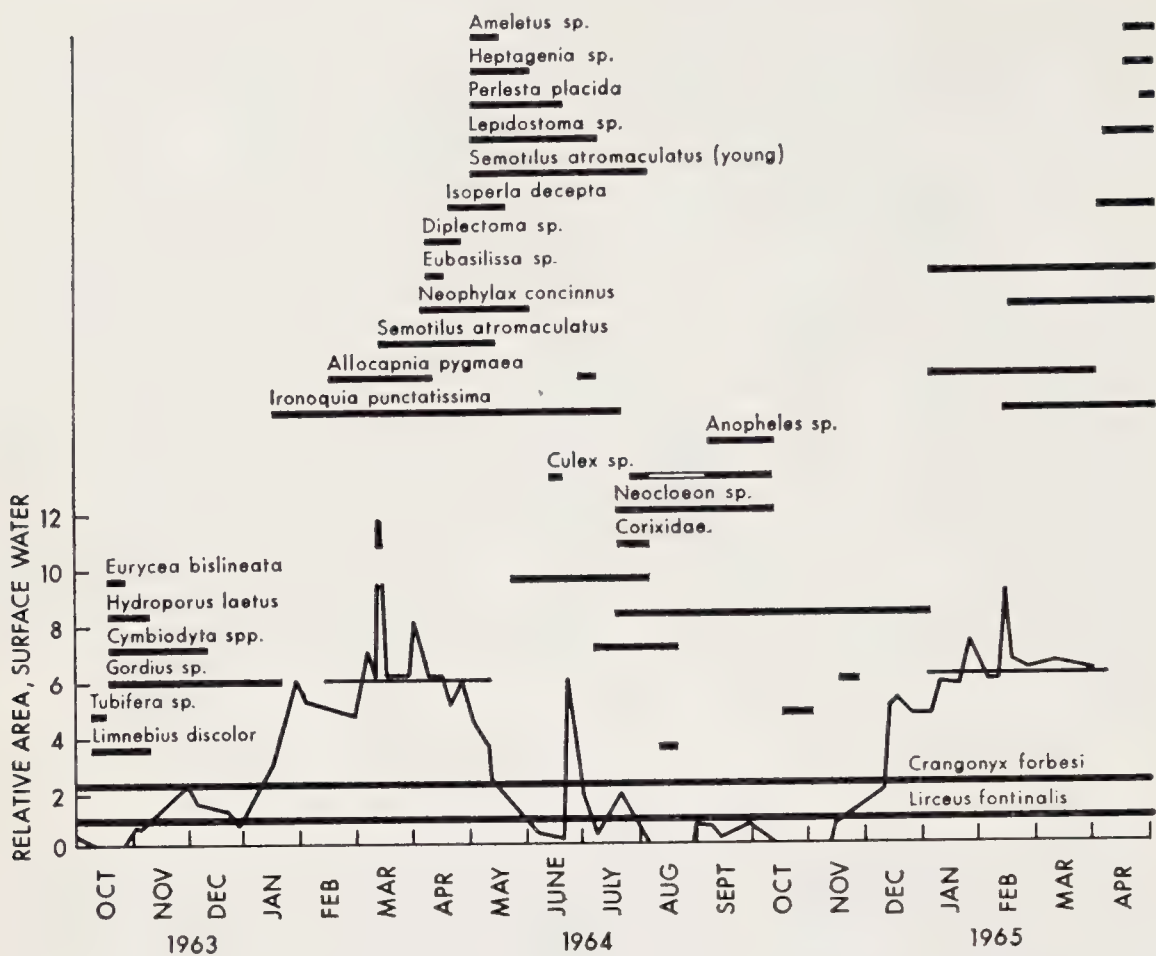


FIG. 3. Stream conditions and seasonal distribution of common invertebrates, 1963-1965. The black bars indicate the months when the various kinds of animals were found active in the stream. All aquatic insects are represented by immature stages with the exception of Coleoptera and Hemiptera, which are reported as adults.

were found mainly from December through February but never in numbers approaching those of the adults. These adult beetles could not be located hibernating during the winter months. Two other true aquatic beetles—*Agabus gasetes* Aube and *Helichus basalis* LeConte (not shown in Fig. 3)—were collected as adults on rare occasions throughout the year. Both were observed hibernating as adults in the winter months. They probably represent the only arthropods of this environment with a life cycle considerably longer than a year.

Tubifera sp., *Culex* spp., and *Anopheles* sp. are adventitious species, which complete their life cycle rapidly whenever standing water is present. A short period of flow during the last week of June 1964 (Fig. 3) temporarily wiped out the population of mosquito larvae. The seasonal distribution patterns of the salamander *Eurycea bislineata*, one of the few vertebrates of the stream, is that of the larvae, the adults being mainly terrestrial. The few remaining pools of July 1964 apparently served as temporary havens for migrating corixids, which disappeared during the dry spell of August, probably moving to more permanent type water. The migratory powers of this group, especially in relation to temporary water, has been documented by Brown (1951).

Gordius sp. were found in considerable numbers during the autumn of 1963 but were rare during the autumn of 1964. The immature stages are parasites of a number of arthropods. It is possible their host, which in this area appears to be chiefly the camel cricket *Ceuthophilus guttulosu*, was not abundant during the autumn of 1964. In October 1963 several specimens were taken from the dry stream bed in soil with little moisture. They were stiff and apparently lifeless, but once removed to water they recovered and became active. Egg masses were found in spring. Depending on the availability of the proper host, the species can complete its life cycle in the intermittent environment.

Neocloeon sp., a baetid mayfly, is a migratory species. The adults emerged from permanent waters, probably Salt Creek, in spring and oviposited in the intermittent stream. Adult females were taken in May 1963. During the first dry spell of August the nymphs, which were small, were able to penetrate deep into moist interstitial spaces and perhaps reach deep subsurface seepage. In October the nymphs were considerably larger although still quite immature as indicated by their wing pads. They were too large to penetrate deep into the stream bed, and they perished during the second dry spell. *Neocloeon* sp. can be considered a migratory species that did not complete its life cycle in Caldwell Hollow.

The winter-spring association is not unlike what might be expected in a permanent stream in the spring. There are five species of caddisfly larvae, three species of stonefly nymphs, and two species of mayfly nymphs. Considering the dry stream conditions in the summer and autumn, it might be expected these aquatic insects are the offspring of early emerging adults that had migrated in from permanent waters. In many instances, however, this is not the case.

Ironoquia (= *Caborius* Navas) *punctatissima* (Walker), a large limniphilid caddisfly, is a striking example of an aquatic invertebrate adapted to an environment that is alternately dry and flooded. Eggs were found in October 1963 under rocks in shaded areas of the dry stream bed. The small larvae appeared in the stream by early January 1964. In May and early June large numbers had congregated in pools that were rapidly drying up. As the pools became completely dry, the larvae and their cases disappeared. It first was thought they had been preyed upon by birds and other vertebrates and did not complete their life cycle. However, Flint (1958), working with another species *I. parvula* (Banks) found in temporary ponds in eastern United States, reported an interesting summer phenomenon. As the pond dried up the larvae actually migrated away from it, aestivated, and finally pupated in leaves near the former high-water line, a habitat which at that time was terrestrial. The pupa of *I. parvula* therefore is truly terrestrial. A terrestrial pupa could explain why Ross (1944, p. 196) never was able to raise successfully *Ironoquia* (= *Caborius*) to the adult using standard aquatic rearing methods. In Caldwell Hollow pupae of *I. punctatissima* have not been found, although larvae apparently aestivating in their cases were located in thick leaf litter to the side of a former pool. That *I. punctatissima* did complete its life cycle is evident from the small larvae that appeared during February 1965.

The larvae of *Lepidostoma* sp., a small leaf-cased caddisfly, disappeared at about the same time and under similar circumstances as *I. punctatissima*. Both species exhibited prepupal larvae during the terminal period of their aquatic life (Table 3). These larvae were shorter and much stockier than their counterparts present in May. They contained large fat deposits, especially in the abdominal segments. Such a condition could be a prelude to impending pupation or a long period of terrestrial aestivation. In respect to the latter, Hinton (1953) reports a correlation between total fat deposit of arthropods and their environment: most aquatic species display less fat than terrestrial relatives, and many species living in environments subject to drought have far more fat than those living in a stable aquatic environment. Lloyd (1921, p. 73) noted fat storage in the caddisfly larva *Neophylax concinnus* McLachlan prior to its long summer aestivation (prepupal) period. *Lepidostoma* sp. also completed its life cycle in the intermittent environment.

Neophylax concinnus is well adapted to life in an intermittent environment. The life history of *N. autumnus* Vorhies has been described

TABLE 3. Number of larvae and size distribution in *Ironoquia punctatissima* and *Lepidostoma* sp., winter and spring 1964. Numbers in heavy type are prepupal larvae = larvae with telescoped segments, sluggish, incapable of eating and probably even walking, exhibiting fat deposits (modified from Lloyd, 1921).

mm	Jan. 27	Mar. 23	Apr. 12	Apr. 23	May 11	May 24	Jun. 1	Jun. 23	Jun. 28	Jul. 5	Jul. 17
<i>Ironoquia punctatissima</i>											
2	2	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—
5	—	5	—	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—	—	—	—
7	—	—	2	1	1	—	—	—	—	—	—
8	—	—	—	1	—	—	1	—	—	—	—
9	—	—	—	2	—	—	—	—	2	—	—
10	—	—	—	1	—	1	—	—	—	—	—
11	—	—	—	2	1	2	—	—	—	—	—
12	—	—	—	1	3	3	—	—	—	—	—
13	—	—	—	—	7	1	—	—	—	—	1
14	—	—	—	1	7	7	5	—	—	—	—
15	—	—	—	1	5	9	—	1	—	—	—
16	—	—	—	1	10	4	3	1	—	—	—
<i>Lepidostoma</i> sp.											
3	—	—	—	—	—	—	—	—	2	—	—
4	—	—	—	—	—	2	—	—	2	—	—
5	—	—	—	—	3	4	—	—	—	1	—
6	—	—	—	—	1	10	2	2	—	—	—
7	—	—	—	—	2	58	17	1	—	—	—
8	—	—	—	—	—	44	34	2	—	—	—
9	—	—	—	—	—	12	3	—	—	—	—

for an intermittent stream (Ross, 1944, pp. 7 and 204) and of *N. concinnus* for permanent streams (Lloyd, 1921, p. 73). Both life histories agree closely with that of *N. concinnus* of Caldwell Hollow. The small stone-cased larvae first appeared in early spring of 1964 in riffle areas. They grew rapidly, and some entered the long prepupal (aestivating) stage in late April when isolated flow still persisted in the stream. They survived part of the dry period in the prepupal stage attached to the under side of rocks, usually in shaded areas. Pupation and emergence took place sometime in September as evidenced by discarded sclerites in abandoned cases. Adults were not found. What apparently were egg masses of this species, as described by Vorhies (1908, p. 671), were located in October and November; however they could not be cultured successfully in the laboratory. *N. concinnus* is a permanent member of the stream community, surviving the early part of the dry period in the prepupal stage, spending a short period as a pupa and adult, and the remainder in a resistant egg stage. Two other caddisflies—*Eubasilissa* sp. and *Diplectrona* sp.—were found during the spring in Caldwell Hollow. Although they were not abundant in either year they apparently are permanent members of the intermittent-stream biota.

Allocapnia pygmaea (Burmeister), a small winter stonefly, emerged from the intermittent stream during March and April 1964. Eggs were laid shortly thereafter and hatched almost immediately. Small nymphs 2 mm in length were taken in subsurface seepage in early July and exhibited little additional development during summer and autumn. In January 1965, nymphs of 2 mm again were found. They apparently resumed development shortly thereafter, as they ranged in size from 4.0 to 5.5 mm by the middle of February and from 5 to 7 mm in the first week of March. At this time emergence began and continued through early April. *A. pygmaea* therefore is a permanent component of the stream, surviving the dry periods as very small nymphs deep in subsurface seepage or moist interstitial spaces. Several stonefly genera including *Allocapnia* have been noted in other North American streams that regularly dry up in summer (Frison, 1929; Gaufin, 1962). Although there is some discussion today as to whether it is the egg or nymph that survives the drought (see discussion p. 143, Gaufin 1962), Frison (1929) demonstrated in his early paper that it was the nymph in *Allocapnia viviparia* (Claassen) and also in a species of *Taeniopteryx*.

Isoperla decepta Frison, a stonefly, and *Heptagenia* sp. (probably *maculipennis* Walsh), a mayfly, were found for very short periods each spring. Both were abundant in 1965 but only *Heptagenia* sp. in 1964 (see Fig. 4). Both emerged during May 1964. The emergence period was synchronous in *Heptagenia* sp., with oviposition occurring sometime during the ensuing 48 hours. Females were observed depositing eggs directly on the surface water of isolated pools. Emergence in *I. decepta* was staggered somewhat. By the middle of May 1965, nymphs were rare, although it was the last week in May before large numbers of females were seen ovipositing. Females did not deposit eggs directly in isolated pools but crawled beneath rocks at the side of pools or even in shallow seepage where water scarcely was visible. Small nymphs of *I. decepta* and *Heptagenia* sp. could not be found during the summer and autumn.

They apparently survive the dry months by having a long hatching period. Still, caution should be used in assigning an egg-resistant stage, especially to *Isoperla* (re. *Allocapnia pygmaea*). Interesting aspects of the life histories of the two species are the very short period when these insects are an obvious part of the stream community and their relatively advanced stage when first found. This is illustrated for *Heptagenia* sp. by Table 4.

It is suggested that both species spend a considerable part of their nymphal life in interstitial water far beneath the surface of the stream bed. This region was adequately sampled in summer and autumn, but little attention was paid to it in spring when the stream was flowing. Chappuis (1942) was one of the first to recognize interstitial water of streams as a distinct habitat, and Orghidan (1959) used the term hyporheic to distinguish it from psammal areas of lakes. Schwoerbel (1961) found stygobiontic water mites to be the most characteristic organisms in this zone, which also served as a "nursery" for various aquatic insects. The stonefly *Leuctra* spent such a large part of its nymphal life in the hyporheic zone that it exhibited a reduction in eyes. In this respect it would be interesting to know the description of the adult.

TABLE 4. Number of *Heptagenia* sp. in each size group during the spring of 1964.

Date	Size (mm)										
	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0
April 25	—	—	—	—	1	—	—	—	—	—	—
May 11	1	1	1	—	7	4	8	7	7	1	3
May 24	—	10	3	10	19	20	11	6	9	1	1
June 1	—	—	—	—	1	—	—	—	4	—	1

A few specimens of *Ameletus lineatus* Traver and *Perlesta placida* (Hagen) were found in the spring months of both years. Little data are available for these species in Caldwell Hollow, although another species of *Ameletus*, *A. ludens* Needham, is one of the few mayflies with a reported history of surviving in streams that regularly dry up (Clemens, 1922). Clemens (1922) also demonstrated in the laboratory a five-month incubation period for eggs of this species, and he believes the long egg stage allows it to exist in intermittent environments. The life history of *A. lineatus* in Caldwell Hollow would appear to be similar. *P. placida* probably has a life cycle similar to *Isoperla decepta*.

The other common animal of the spring fauna was the creek chub, *Semotilus atromaculatus*. It migrated into the stream from Salt Creek during high water of March 1964. Reproduction took place here, but the young perished during the first dry period of August, and hence it was unable to complete its life cycle in the intermittent environment. Its absence in the spring of 1965 was due to rotenone poisoning of Salt Creek during the fall of 1964.

Tricladids including probably *Phagocata morgani* (Stevens and Boring) were common macro-invertebrates of the stream, but were not adequately sampled. Organisms present on few occasions included the caddisflies: *Drusinus* sp. and *Wormaldia* sp. (both in spring); mayflies: *Siphonurus* sp. and *Paraleptophlebia* sp. (spring); dragonfly: *Cordulegaster obiquus* Say (autumn); coleopteran: *Helichus fastigiatus* (Say) (non-seasonal); dipterans: *Tabanus* sp. and Tipulidae (autumn); malacostracans: *Orconectes propinquus* (Girard) and *Asellus stygius* (Packard) (both non-seasonal); vertebrates: *Plethodon dorsalis* (winter) and *Notropis cornutus* (spring). Entomostracans and nematodes were not investigated. Molluscs were not present.

There were striking differences between the spring fauna of Caldwell Hollow and that of adjacent permanent streams in 1964. In the latter, *Drusinus* sp. *Ptilostomis* sp., hydropsychids, *Wormaldia* sp., *Leptophlebia* spp., *Stenonema* spp., *Baetis* spp., *Agrion* sp., *Psephenus* sp., and various species of Tendipedidae were common in spring and early summer. Large numbers of *Lirceus fontinalis* and a few *Crangonyx forbesi* also were found in March and April in the permanent streams. The situation was changed in the spring of 1965, due chiefly, it is believed, to the rotenoning of Salt Creek and its permanent tributaries in the autumn of 1964. Invertebrates in Salt Creek were sparse and consisted mainly of species that also were present in Caldwell Hollow. Chief among these were *L. fontinalis*, *Heptagenia* sp. (same species as Caldwell Hollow), *Ameletus lineatus*, and *Isoperla decepta*. It seems likely that small streams such as Caldwell Hollow, which were completely dry at the time of the poisoning, contributed substantially to the initial reestablishment of invertebrates in the poisoned stream.

The percentage composition of major invertebrates throughout the year (Fig. 4) reflects the overall dominance of the two malacostracans. With the exception of April and May when the spring fauna was well represented, *L. fontinalis* and *C. forbesi* consistently made up 80 to 90% of the total number of organisms. During June, July, and part of August, all specimens of *C. forbesi* were very small—in the 2 and 3 mm size range. They were present in deep subsurface water and moist interstitial spaces but were not quantitatively sampled.

From the above information it is possible to obtain a fairly accurate picture of similarities and differences between the fauna of this intermittent stream and that of permanent streams. Caldwell Hollow is characterized by the two crustaceans, *L. fontinalis* and *C. forbesi*, which survive the dry months as very small organisms in deep subsurface seepage and even moist interstitial spaces. All remarks must be made relative to their dominant position. Invertebrates active in the spring are mostly permanent residents of the stream. The same species appeared in both years, constituting a uniform and apparently stable community. This is in contrast to drought-stricken permanent streams or even streams that regularly are reduced to isolated pools in the summer. Here the studies of several workers (Hynes, 1958; Larimore *et al.*, 1959; and Sprules, 1947) demonstrate the immediate destruction of many invertebrates under such adverse conditions.

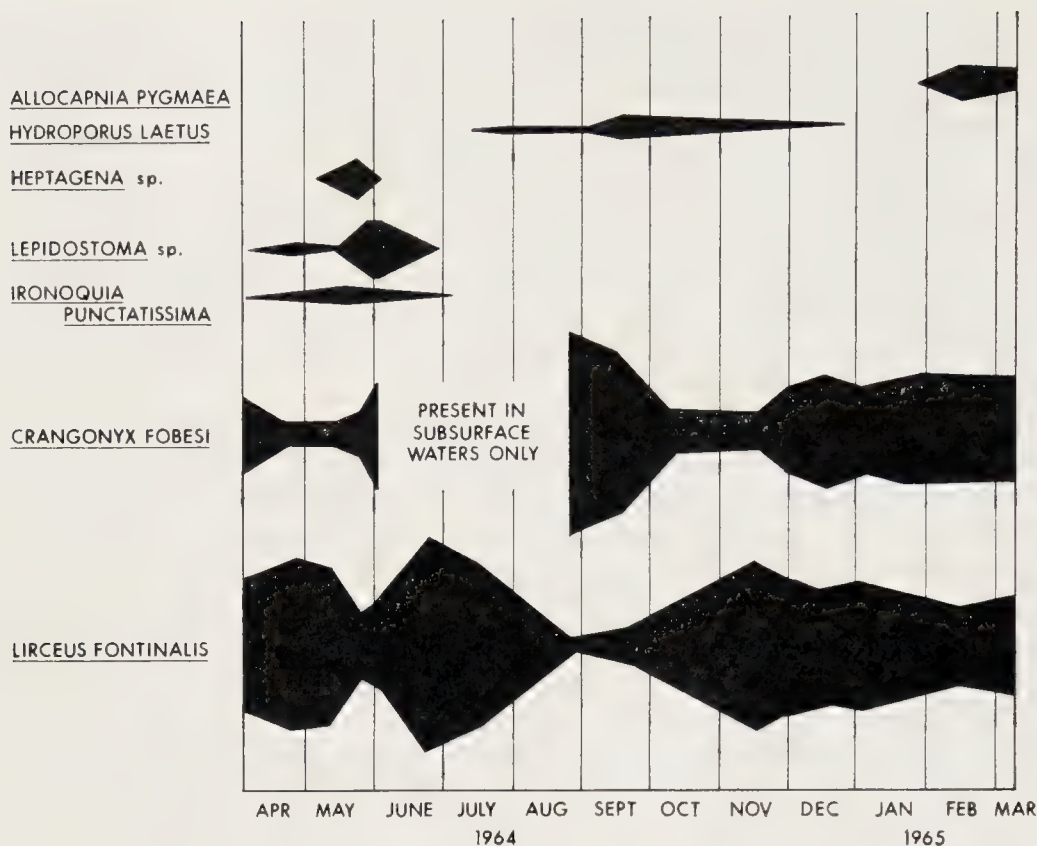


FIG. 4. The percentage composition throughout the year of invertebrates that made up at least 2% of the total monthly sample. The width of the spindle is proportional to the number of specimens taken on the sampling date.

The active spring fauna of Caldwell Hollow survive the dry periods in diverse ways, all of which are associated with favorable stages of their life cycle in relation to drought conditions. The holometabolous caddisflies appear to be the most highly adapted of the aquatic insects, surviving the dry periods as large aestivating larvae or pupae in at least a semiterrestrial habitat. Mayflies and stoneflies survive the dry spells as very small nymphs exhibiting no growth during these periods, or as eggs. All the members of the spring fauna had only one generation a year (were univoltine), exhibited little growth in the summer, and completed their life cycles in approximately one year (had annual cycles). Also, all the aquatic insects with the exception of caddisflies emerged in the spring. The regularly occurring dry periods, therefore, eliminate (1) bi- or multivoltine invertebrates, *e.g.*, *Baetis* spp., *Stenonema* spp., and *Neocloeon* sp.; (2) animals with life cycles of two or more years, *e.g.*, *Pteronarcys* spp., and *Acroncuria* spp.; (3) animals with a major growth period in summer and an emergence in autumn, *e.g.*, *Hydropsyche* spp. and *Ephemerella* sp.

The above mentioned criteria characteristic of the intermittent-stream fauna are also shared by a variety of permanent-stream inhabitants. Hynes (1961) for a Welsh mountain stream reports at least eight species of mayflies and stoneflies whose life histories would seem to qualify them nicely for the intermittent environment. Other examples

could be cited. Of course the intermittent-stream organisms themselves are found also in permanent waters. With the exception of the caddisflies, especially *I. punctatissima*, *Lepidostoma* sp., and *N. concinnus*, the species are not specifically adapted to the intermittent environment of Caldwell Hollow but are able to survive there by utilizing favorable aspects of their life cycles.

REPRODUCTIVE CYCLES

OF *Lirceus fontinalis* AND *Crangonyx forbesi*

The previous discussion suggests that the life cycles of permanent univoltine species in intermittent streams can be considered in most instances "pre-adapted" rather than adapted *per se* to the adverse conditions of this habitat. Moreover, the number of the non-seasonal crustaceans, *L. fontinalis* and *C. forbesi*, far exceeds that of the seasonal fauna. The following sections are concerned with factors that may be related to the dominant position of the two malacostracans in the intermittent environment.

The specific facts of reproduction for aquatic amphipods and isopods, respectively, are reviewed by Hynes (1955) and Ellis (1961). Female peracarid crustaceans are characterized by the possession of a brood pouch, or marsupium, in which the eggs are carried and the young undergo development. In brief, the female molts releasing the oostegites to form the brood pouch. Copulation ensues, with or without a period of precopula, and shortly thereafter the eggs are released into the pouch. After a period of days the young develop and are released from the pouch. The females of most species may mate again.

Lirceus fontinalis

With reference to aquatic isopods, *L. fontinalis* in a permanent stream of Illinois reproduces throughout the year but with a peak in the spring months (Marcus, 1930). Minckley (1963) also observed reproduction in *L. fontinalis* throughout the year in a Kentucky stream. In the same stream another isopod, *Asellus bivittatus* Walker, breeds in every month, with a peak in the spring. Ellis (1961) reports gravid *A. intermedius* Forbes in a Michigan trout stream during every month, although few in winter. In an Illinois stream that is reduced to isolated pools in the summer, Allee (1912) noted breeding peaks in April and late fall for *A. communis* Say, with occasional breeding during the summer but very rarely in August and September. Of the brackish water isopods, *Idotea emarginata* (Fabricius) breeds throughout the year in Great Britain (Naylor, 1955). The trend therefore, is for temperate aquatic isopods to reproduce throughout the year, with peaks in the spring months.

In contrast, the period of reproduction of *L. fontinalis* in Caldwell Hollow is restricted to late winter and spring (Fig. 5). Laboratory experiments with three pairs of animals indicate that each female has but one brood during its reproductive phase, with an average incubation of 33 days at 13° C. Assuming this is also the situation in nature, the

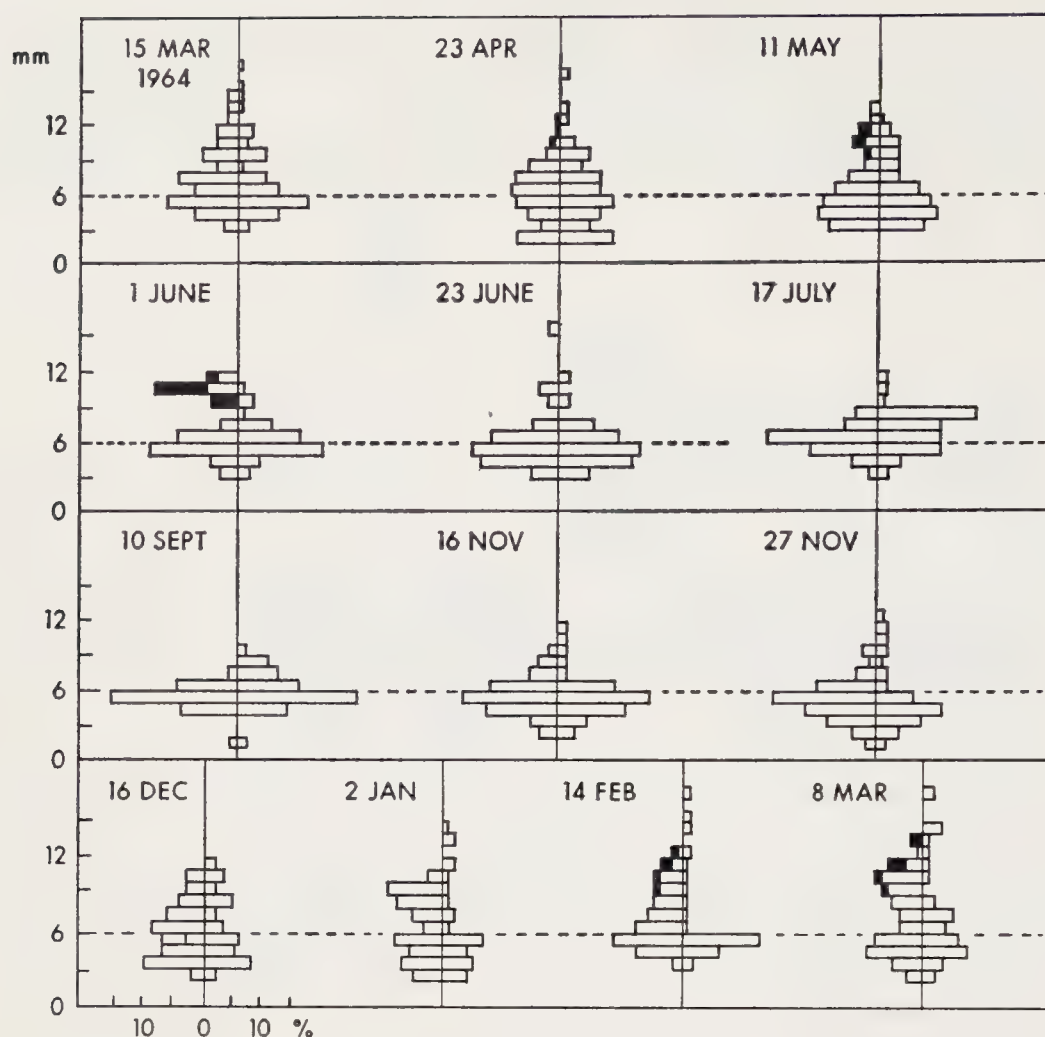


FIG. 5. Size and reproductive condition of *Lirceus fontinalis*. Juveniles are centered below the dotted line. Above this line females are on the left, males on the right of the midline. Females with brood pouches are blackened.

life cycle of *L. fontinalis* in the intermittent environment may be described as follows.

In Caldwell Hollow in 1964 and 1965 there was an annual cycle with a life span of approximately one year. The first of the large overwintering adult females became ovigerous in late March and early April 1964 (Fig. 5). With a maximum incubation time of 30 days (the water temperatures averaged 15°C at this time), the first of the new generation should have appeared in late April and early May, although the very small animals of 1 and 2 mm were not adequately sampled. This new generation grew rapidly, and some were sexually differentiated by June. Meanwhile, overwintering juveniles and smaller females had grown and became ovigerous in late May and June. Their offspring would not appear until late June. The two peaks of ovigerous females are reflected by the percentage of females with young and with empty pouches during the reproductive period and by the paucity of ovigerous specimens in the middle of the

TABLE 5. *Lirceus fontinalis*. Summary of samples collected for studying the reproductive cycle.

Sampling date	1964										1965		
	Mar. 15	Apr. 23	May 11	Jun. 1	Jun. 23	Jul. 17	Sep. 10	Nov. 16	Nov. 27	Dec. 16	Jan. 2	Feb. 14	Mar. 8
No. of specimens	190	271	351	187	152	112	83	145	115	134	125	153	153
% juveniles (less than 7 mm in length)	54	48	54	42	60	29	61	66	49	48	43	39	29
% males	19	22	23	23	21	44	28	15	19	30	25	27	32
% females* without brood pouches	27	27	18	22	19	27	11	19	32	22	32	30	30
% ovigerous females	0	3	1	5	0	0	0	0	0	0	0	4	7
% females with young	0	0	2	0	0	0	0	0	0	0	0	0	0
% females with empty brood pouches	0	0	2	8	0	0	0	0	0	0	0	0	2
Av. length of ovigerous females (mm)	—	12.0	12.5	11.0	—	—	—	—	—	—	—	12.5	12.5
Range of eggs or embryos in brood pouches	—	180-270	130-200	110-180	—	—	—	—	—	—	—	40-275	170-270

* with or without oostegites

period (Table 5). All overwintering animals, both males and females, had perished by the end of July. The new generations grew very little in the summer and early autumn when temperatures were high and the stream was periodically dry. It was during this time that the animals, because of their small size, were able to penetrate deep into the moist interstitial spaces of the stream bed.

In September, prior to the resumption of rapid growth, there were essentially only two size groups. One consisted chiefly of juveniles 4-6 mm long, the offspring of the early reproducing adults of the previous spring. The other group consisted of very small animals approximately 2 mm in length, resulting from the late reproducing females of 1964. Failure of reproduction to occur in late summer and fall is due to the lack of mature animals at these times. This in turn is influenced by the arrested growth of the new generation during July, August, and September and also possibly by the early mortality (due to drought) of still potentially reproductive animals of the old generation.

Growth through the winter was rapid. The first females with small oostegites appeared in the middle of December, and by 14 February the first ovigerous females of 1965 were present. These females and their larger male counterparts, therefore, probably derive from the early reproducing adults of 1964.

Factors important for the survival of *L. fontinalis* in the intermittent stream are the small size of most specimens when the stream initially goes dry and the following period of arrested growth. Since it has been determined that most aquatic isopods have a peak vernal period of reproduction, size in itself can not be considered an adaptation to the intermittent environment. Arrested growth during adverse conditions has been reported for a variety of animals. In some instances this phenomenon is reportedly due to external environmental elements and in other cases to internal or intrinsic factors.

Some obvious exogenous components to be considered in the intermittent stream during the dry period are high temperatures and decreased availability of oxygen. Both these conditions are realized in Marcus' (1930) permanent stream during the summer months where *L. fontinalis* grew and reproduced throughout the year. Hence, these factors alone are not adequate to explain the situation in Caldwell Hollow, and it is suggested that an equally important factor is photoperiodicity. The small animals survive drought periods deep beneath the stream bed, receiving little if any light until the water table again builds up in the autumn, at which time rapid growth again resumes. Stephens (1955) working with a decapod crustacean, *Cambarus virilis*, found an increased tendency for it to molt with increasing length of photoperiod. Those kept in complete darkness for 64 days failed to molt. Tempting as this hypothesis is, its verification for *L. fontinalis* would have to rest on detailed study beyond the scope of the present investigation.

Egg production was not studied in detail. The wide range in number of eggs in the brood pouch (Table 5) reflects failure of eggs to develop, capture when all eggs were not fully laid into the pouch, varying size of females (since larger females may average more eggs than smaller ones), and in some instances loss from the pouch because the eggs were

not counted immediately after collection. The females with empty pouches of 8 March 1965 exhibited opaque oostegites indicating they had just molted to the mating condition, as confirmed by the dissection of eggs from the ovaries. The average number of eggs (no cleavage), embryos (somites apparent), and young (eyes apparent) was 220, 105, and 55 respectively. These data are from females throughout the reproductive period regardless of time and size. This disparity undoubtedly reflects some mechanical loss of late-stage embryos and young when stored in alcohol for long periods and also possibly early expulsion of these stages from crowded brood pouches. That it also indicates failure of some eggs to develop was demonstrated by the presence of many uncleaved eggs in the brood pouches of females when all other "offspring" were in a late embryo stage. Ellis (1961) found only 17% mortality from egg to young in *Asellus intermedius*, while Jancke, as reported by Ellis (1961), observed almost 50% mortality in *Asellus aquaticus*.

Crangonyx forbesi

In contrast to aquatic isopods, temperate aquatic amphipods usually do not reproduce throughout the entire year. Exceptions are *Gammarus minus* Say of eastern United States (Bousfield, 1958) and *Crangonyx pseudogracilis* Bousfield (= *gracilis* Smith *partim*) in Great Britain (Hynes, 1955). Although some species may have generations in the spring and autumn, such as *G. fasciatus* Say (Clemens, 1950; Hynes, 1955) and *Hyalella azteca* (Saussure) (Gaylor, 1921), most have only one generation a year. Reproduction in North American species is usually restricted to spring and early summer, with life spans of 12 to 15 months. The deep-water northern species *Pontoporeia affinis* Lindstrom is an exception, breeding during the late autumn and winter and having a life span of approximately two years (Larkin, 1948). Bousfield (1958) also indicates a winter breeding season for the northern species *Paramoera*. Little is known of reproduction in *Synurella* Wrzesniewski, *Bactrurus* W. P. Hay, and the various subterranean genera common in south-central United States.

Bousfield (1958) reports ovigerous *C. forbesi* from March to May, and this agrees with the results reported here. Ovigerous females first appeared in early April (Fig. 6), although spot collections indicated their presence in the middle of March. By the last of April ovigerous specimens had reached their peak, and in early May the new generation was present. Shortly thereafter egg production declined (Table 6). The overwintering animals started to decline in abundance during May, a time when many pools still persisted in the stream. Mature males are smaller than females in this species, and there is indication they die before the females. Sometime during June the last of the 1964 reproducing generation perished. The new generation disappeared from the few remaining pools in June and was found deep in subsurface seepage which could be located through August. An adequate sample could not be obtained from 1 June to 25 August.

As was found for *Lirceus*, growth through the summer and early autumn was slow. By November more rapid growth was taking place, and the first females were distinguishable. Females, being larger, could

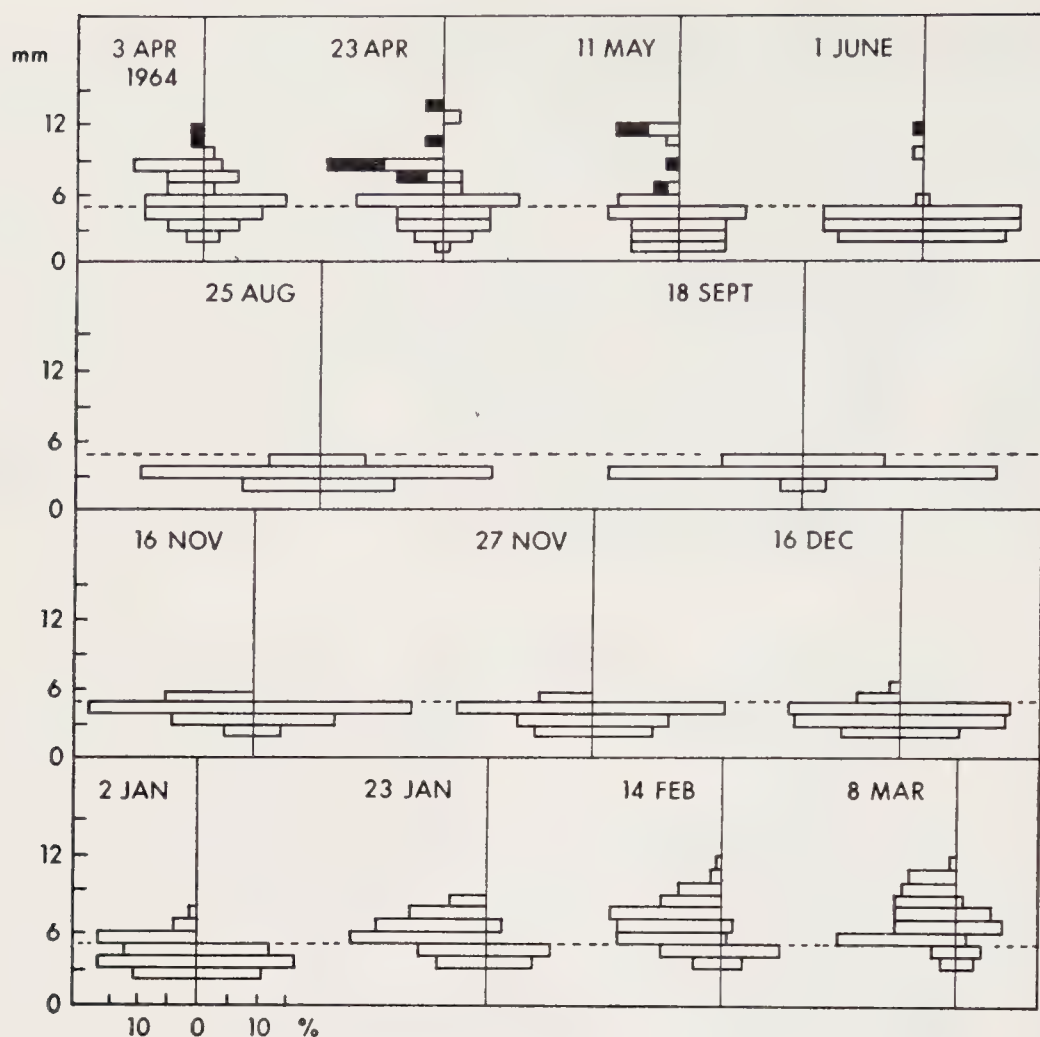


FIG. 6. Size and reproductive condition of *Crangonyx forbesi*. Juveniles are centered below the dotted line. Above this line females are on the left, males on the right of the midline. Females with brood pouches are blackened.

be recognized before males, which were not distinguished until late January. The first ovigerous female of 1965 was collected on 19 March (not indicated in Fig. 6).

Because of small although representative samples in the initial phase of the study, information on egg production is sketchy. The average number of eggs per female was 90, and although culture experiments are inconclusive, it appears that each female produces only one brood and then dies. Of six laboratory pairs, only one female became ovigerous, and it perished before carrying the brood to "term." Females that were ovigerous when brought into the laboratory produced only one brood. The decrease in size of ovigerous specimens through the reproductive period (Table 6) lends credence to the view that each female produces only one brood and then dies. Death of the adult after release of young from the brood pouch has been reported for two other species of *Crangonyx* (Bousfield, 1958, pp. 94 and 96).

TABLE 6. *Crangonyx forbesi*. Summary of samples collected for the study of reproductive cycle.

Sampling date	1964										1965		
	Apr. 3	Apr. 23	May 11	Jun. 1	Aug. 25	Sep. 18	Nov. 16	Nov. 27	Dec. 16	Jan. 2	Jan. 23	Feb. 14	Mar. 8
No. of specimens	53	40	61	88	400	300	33	68	141	88	69	217	160
% juveniles (less than 6 mm in length)	38	33	69	96	100	100	85	91	92	78	37	27	14
% males	24	17	—	1	—	—	—	—	—	—	3	3	17
% females* without brood pouches	34	30	21	2	—	—	15	9	8	22	60	70	69
% ovigerous females	4	12	2	—	—	—	—	—	—	—	—	—	—
% females with young	0	5	3	0	—	—	—	—	—	—	—	—	—
% females with empty brood pouches	0	3	5	1	—	—	—	—	—	—	—	—	—
Av. length of ovigerous females (mm)	12	9	8	—	—	—	—	—	—	—	—	—	—
Range of eggs or embryos in brood pouches	85- 93	55- 98	35	—	—	—	—	—	—	—	—	—	—

* with or without oostegites

It previously was pointed out that males die before the females. Since the 1965 winter sample gives no indication that males are born earlier in the season than females (the situation found in *Gammarus duebeni* Lillj. by Kinne, as reported by Hynes, 1955), it is likely they have a shorter life span than females. The more numerous females during early growth of the new generation (December, January 1965) can be explained by the larger females reaching 5 mm in length (below which all animals were considered juveniles) before males. This disparity, however, carries through the reproductive season. If April is considered the peak month of reproduction in *C. forbesi*, there is still a sex ratio of 2.2 females to 1 male. Although caution must be used in making inferences from the small April samples, it seems there may well be a preponderance of females in this species.

Generalized life cycles

From above data on *L. fontinalis* and *C. forbesi* it now is possible to describe their life histories and the significance of their reproductive cycles in relation to the intermittent environment. Late autumn and winter, when temperatures are low and the water table is building up, is a period of rapid growth for juveniles and young adults of both species. They begin to breed in March and April, months that normally exhibit the maximum amount of water in the intermittent stream. Breeding is restricted to the vernal period for both species. In *L. fontinalis* its termination appears to be facultative in part, due to the early death of still potentially reproductive animals of the old generation and the arrested growth of the new generation. Both phenomena are influenced by the onset of the dry period. In *C. forbesi*, at least in this region, reproduction unvaryingly occurs only in the spring, with adult females producing one relatively large brood and then dying prior to the drying up of the stream. All the old generation of both species perish by June and July, a time when few pools remain. The individuals of the new generations grow very little during summer and early fall when the stream is completely dry or consists only of transitory pools. The very small animals survive deep in subsurface seepage or, as will be pointed out in a later section, in moist soil *per se*. With the formation of permanent pools in late fall and influenced by falling water temperatures and possibly other factors, such as photoperiod, rapid growth resumes.

SEASONAL OXYGEN CONSUMPTION, *L. fontinalis*

It is apparent that *L. fontinalis* and *C. forbesi* exhibit little growth in summer and early autumn (Figs. 5 and 6), which are the times when the stream is dry or consists only of transitory pools. Water that is present has a low O₂ content and high temperatures (Fig. 2). Aquatic poikilotherms during this time must adjust in some way to these surroundings in order to survive. One such adaptation could be a reduced metabolic rate in summer and early autumn. Metabolic rate as used here refers to oxygen consumption per unit body weight per hour, in contrast to metabolism, which refers to total oxygen consumption of the animal per hour (after Zeuthen, 1953).

Yearly variations of metabolic rate in aquatic invertebrates have not been investigated extensively. Most data that are available have been reported only on a bi-yearly basis (*e.g.*, winter and summer). In respect to crustaceans, Edwards and Irving (1943a), working with the sand crab *Emerita talpoida* Say, found oxygen consumption four times higher in winter than in summer when measured at 30°C. The higher metabolic rate in winter was interpreted as a winter adjustment sustaining growth and activity at low temperatures. Krog (1954) obtained evidence of a lower oxygen consumption in winter than in summer for an amphipod from Alaska, *Gammarus limnaeus* (Smith), which he associated with the low oxygen content of the ice-covered lake in winter. Roberts (1957), working with the shore crab *Pachygrapsus crassipes* Randall, reported a parallelism between metabolic rate and short-term fluctuations of local temperatures in southern California but found no definite seasonal trend. Clark (1955) reported the oxygen consumption of a soil-inhabiting amphipod of the Australian rain forest, *Talitrus sylvaticus* (Haswell), to be significantly higher in winter (*e.g.*, July) than in summer (*e.g.*, January). In contrast, the amphipod *Talorchestia megalopthalma*, which inhabits the ocean beaches of eastern United States, was found to have the same metabolic rate in winter and in summer (Edwards and Irving, 1943b). However, animals of the same body size were not used throughout this study. By recalculating the data from the above study and taking into consideration body size, Rao and Bullock (1954) also projected a higher winter metabolism for *T. megalopthalma*. Frankenberg and Burbanck (1963) found no significant change during the year in the oxygen consumption of the estuarine isopod, *Cyathura polite* (Stimpson), when compared between November and March and between May and July. There are numerous reports and reviews in the literature of other compensatory mechanisms related to oxygen consumption of aquatic poikilotherms, including crustaceans. Macan (1961, pp. 170-179) reviews many of these factors in relation to ecology.

For *L. fontinalis* in Caldwell Hollow, oxygen consumption declined in late spring and summer when measured at 5° C (Fig. 7). A corresponding decrease was not evident at 13° C. However, when comparing oxygen consumption on a yearly basis, individuals in the same physiological state should be used (*e.g.*, age, sexual state, size, activity, etc.). Because of its restricted reproductive period and the early death of still mature specimens, this obviously was impossible for *L. fontinalis*. The best that could be hoped for was to use animals of uniform body size throughout the study period, but even this was impossible (Fig. 7), because only small animals were present in summer and early autumn. Although the relation of metabolic rate to body size was realized, no correction for body size was made in this study. A winter weight-specific regression coefficient of -0.29 was estimated at 13° C for *L. fontinalis*. This figure agrees closely with those of other isopods—*Asellus aquaticus* L., -0.32 at 10° C (Edwards and Learner, 1960) and *Ligia oceanica* L., -0.27 at 25° C (Ellenby, 1951). If the regression line has the same slope in all seasons, a factor not investigated, the animals at 13° C also would exhibit a lower metabolic rate in summer, when size is considered.

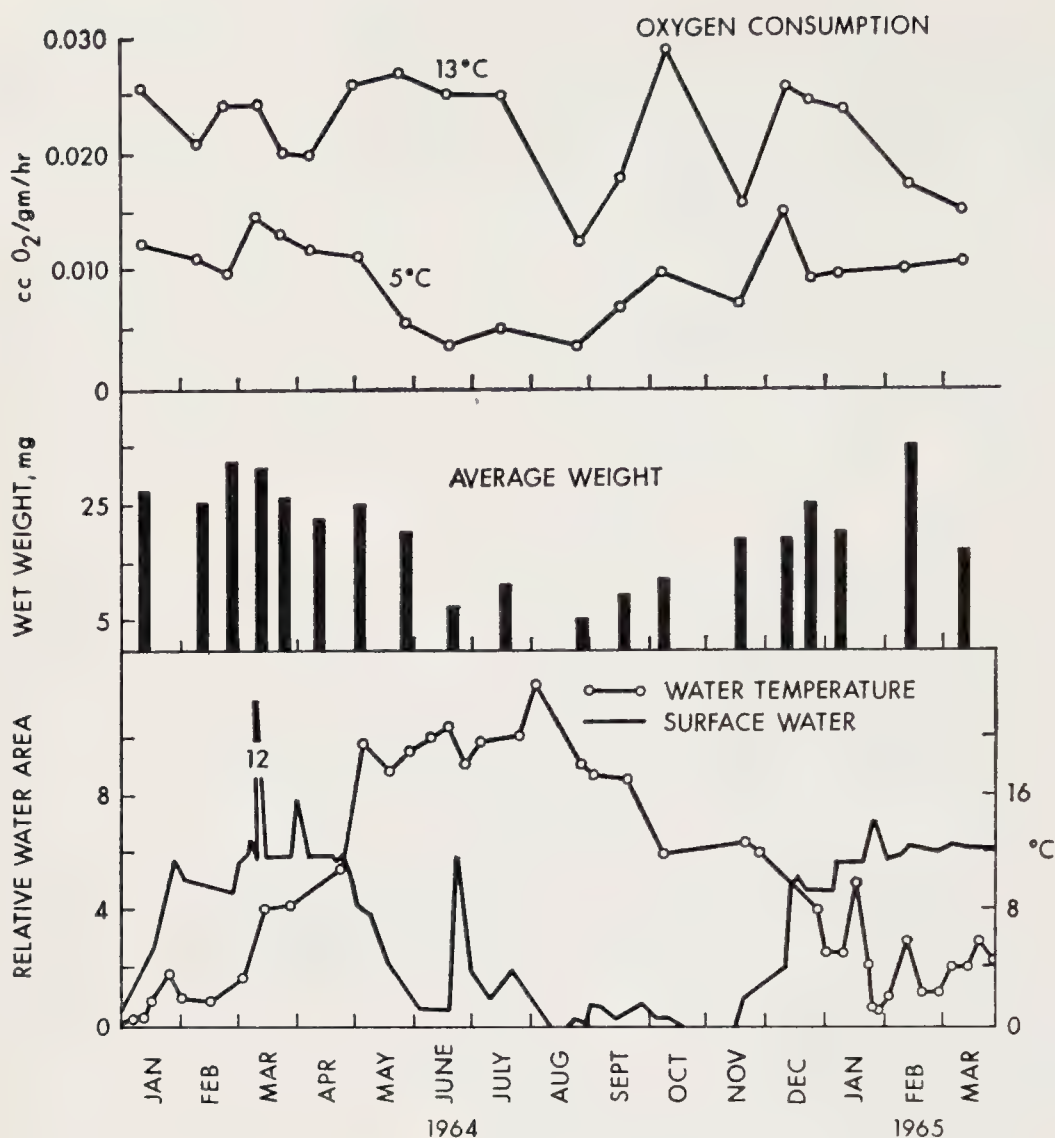


FIG. 7. Oxygen consumption of *Lirceus fontinalis*, water temperature, and visible surface water, January 1964-March 1965.

The metabolic rate at 5° C declined rapidly in May, at which time isolated pools were drying up, and water temperatures were becoming high (Fig. 7). Dissolved oxygen also was becoming depleted (Fig. 2). Temperatures started declining in August and September, and it was at this time that the oxygen consumption of *L. fontinalis* began to increase. No connection could be established between this initial autumn increase and any other changes occurring in the aquatic habitat at this time. The stream was almost completely dry, and dissolved oxygen was extremely low. Hence, the seasonal changes in metabolic rate seem related neither to regularly occurring changes in the water regime nor to changes in dissolved oxygen content. The seasonal changes follow closely but inversely that of temperature.

In this respect the lower metabolic rate of summer animals at 5° C may be due in part to a cold-depression effect, in that animals taken from their warm habitat and transferred to 5° C may have been depressed

at this temperature even though they were acclimated to the test temperature. A temperature of 5° C would not depress winter animals.

An increase in oxygen consumption during the reproductive period has been reported for other invertebrates, especially molluscs (Bruce, 1926; Berg *et al.*, 1958). Knowing the life cycle of *L. fontinalis* in Caldwell Hollow it is possible to compare the oxygen consumption of reproductive and nonreproductive individuals. Animals approximately 10 mm in length, which is a sexually mature size for this species, were collected in winter and spring. No ovigerous females were used, however. The reproductive period of *L. fontinalis* extended from April to early June in 1964 (Fig. 5), but maximum oxygen consumption (5° C) appeared to be reached in March. It was declining rapidly in May, the middle of the reproductive period. If there is a connection between metabolic rate and reproduction, it was masked by other stronger compensatory factors such as temperature.

As previously mentioned, *L. fontinalis* (and *C. forbesi*) grow little during the summer. Whether or not the physiological processes are such that the animals might be considered in a state of aestivation during this period is not definitely known. Certainly, aestivation might be a process by which the animals could survive the unfavorable summer conditions, and the lower summer metabolic rate suggests such a possibility. Garten-Fischler (as reported by Eckstein and Abraham, 1959) obtained evidence of a depressed oxygen uptake in aestivating snails. In this respect it would be interesting to measure monthly oxygen consumption of *L. fontinalis* maintained at a constant temperature throughout the year to see if there is an intrinsic lowering of the metabolic rate in summer. This very phenomenon has been observed for a species of killifish (Wells, 1935).

HUMIDITY AND SOIL FACTORS

Humidity and survival of L. fontinalis

The only true terrestrial crustaceans, in the sense of living and reproducing without immersion in water, are found in the same order to which *L. fontinalis* belongs, such as the numerous species of terrestrial isopods commonly known as sow bugs or pill bugs. These organisms survive in air by occupying areas of high relative humidity. The terrestrial isopods can be arranged in a series with respect to their powers of water retention and their habitat preference. *Ligia* is least adapted to terrestrial environments, being found close to water and exhibiting low water-retention abilities. *Oniscus* is intermediate in habitat preference and water retention, while *Armadillidium* lives in drier places and has greater water-retention capacities. Recently Warburg (1965) reported a desert isopod, *Venezillo arizonicus* (Mulaik and Mulaik), with very high water-retention abilities. Lagerspetz and Lehtonen (1961) and Lagerspetz (1963) in Finland conducted a series of humidity experiments using true aquatic isopods and amphipods. Those species that lived the longest time in water-saturated air (*Asellus aquaticus* and *Gammarus duebeni*) were the same species found in temporary brackish water pools.

An experiment, using methods similar to those of Lagerspetz, was carried out with *L. fontinalis* and *C. forbesi* from the intermittent stream and with *L. fontinalis* and *Gammarus minus* from a permanent stream. Animals were blotted carefully and placed on the bottom of open, dry plastic containers. These in turn were placed in a humidity chamber containing water-saturated air at 13° C. The humidity chamber was prepared by placing water in the bottom of a desiccator. Strips of filter paper lined the walls and extended into the water. Shortly after the lid was placed on the desiccator, the air in the chamber was saturated with water vapor, a condition not unlike what might be expected in deep interstitial spaces of the otherwise dry stream bed. Animals were removed from the humidity chamber for approximately 30 seconds each day and were given tactile stimulation to determine if they still were alive. Those that did not respond were removed to water, but none recovered. After death, the animals were measured and their wet weight extrapolated from a wet weight-total length graph of non-dehydrated specimens. No correlation between body size and survival time was apparent within a species. The experiment was conducted during February and March, a time when the metabolic rate of *L. fontinalis* from Caldwell Hollow was high (Fig. 7).

Both *L. fontinalis* and *C. forbesi* from the intermittent stream lived for a longer period of time than did the organisms from the permanent stream (Fig. 8). The last specimen of *L. fontinalis* from Caldwell Hollow survived until the twenty-fourth day. By this time three of the five control animals that were in water but without food also had died. Hence, it is possible that this animal died of starvation instead of a water loss or an inadequate oxygen supply. The longer survival time of *Lirceus* from the intermittent environment when compared to the same species from the permanent stream may be ecotypic. Specimens from the permanent habitat also reached their reproductive peak at an earlier time (January and February). *G. minus*, which had the shortest survival time, never has been found in intermittent streams from this area. Ten mature nymphs of *Allocaenia pygmaea* also were tested for survival. At the end of 24 hours, eight had died and one had emerged. The remaining nymph perished prior to 48 hours.

A comparison of Lagerspetz's results with those reported here (Table 7) is complicated somewhat by different test temperatures. The work in Finland was performed at 19° C, while the above experiment with organisms from Caldwell Hollow and Leonard Spring was conducted at 13° C. It also should be pointed out that *L. fontinalis* and *C. forbesi* are larger species than *A. aquaticus* and *G. duebeni*. Even considering these differences, and of course both experiments were performed with no saturation deficit, it appears as if *L. fontinalis* is better adapted than *A. aquaticus* for longer survival periods in moist soil. The amphipod data are rather similar.

In respect to *G. duebeni*, several Scandinavian workers including Segerstrale (1946, p. 18) have observed the species moving from pool to pool via the terrestrial environment. No evidence of such migrations could be found for *L. fontinalis* or *C. forbesi* in Caldwell Hollow. However, specimens of *L. fontinalis* were observed close to the surface of the dry stream bed during May and early June when widely isolated pools still

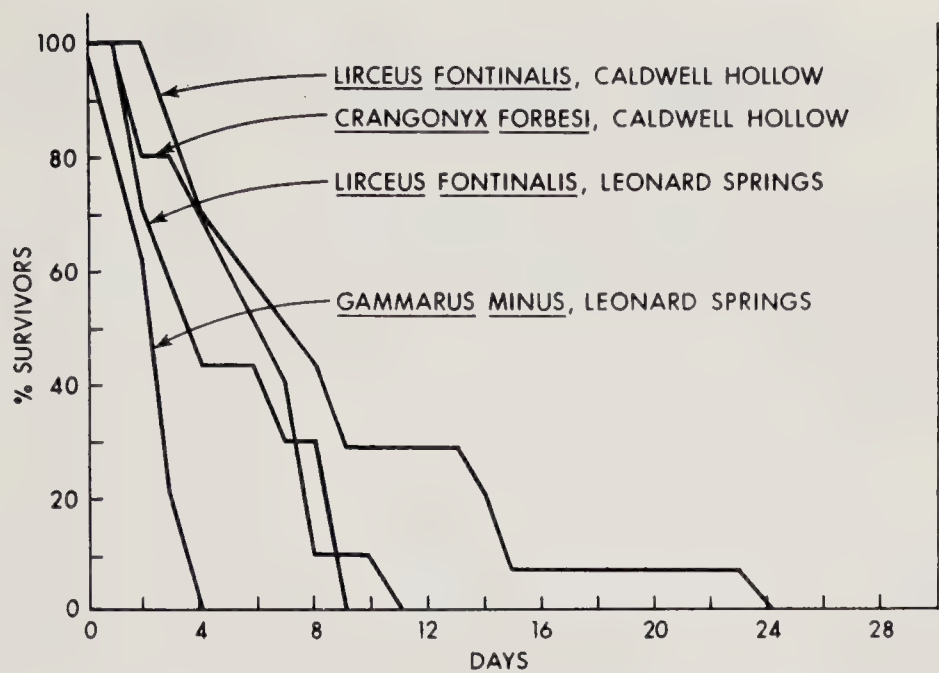


FIG. 8. Percentage survival of intermittent and permanent stream organisms in water-saturated air (100% relative humidity).

were present and subsurface seepage between these pools was rather high in the stream bed. Such near-surface occurrences, which suggest vertical migration to the surface of the stream bed, were observed only at night when atmospheric humidity was high. Hence, even though no animals were actually observed traveling up or down the dry stream bed, this would not seem an impossible task considering their survival time in water-saturated air and similar conditions that exist in the stream at certain times.

The ability to survive in moist soil would have special significance for the survival of large animals during short dry periods, *e.g.*, March

TABLE 7. Maximum and 50% survival time of intermittent-pool crustaceans from Finland and intermittent-stream crustaceans from Caldwell Hollow, Indiana, in water-saturated air at 19° C and 13° C, respectively.

	Survival time 50% of animals (days)	Maximum survival time (days)
Amphipoda		
<i>Gammarus duebeni</i> (Finland)*	7	9½
<i>Crangonyx forbesi</i> (Indiana)	6	11
Isopoda		
<i>Asellus aquaticus</i> (Finland)†	1¼	3
<i>Lirceus fontinalis</i> (Indiana)	7	24

* Lagerspetz (1963)
† Lagerspetz and Lehtonen (1961)

and April. The animals would be too large to follow the subsurface seepage downward to any appreciable depth but could survive for short periods in larger interstitial spaces containing water-saturated air.

Soil samples

In respect to the terrestrial environment it is interesting to examine more closely data collected when the stream bed was either partially or entirely dry (Table 8). Each soil sample collected contained approximately 400 cc of material. The samples were separated into the following, somewhat subjective categories.

a) *Subsurface seepage*. Sand, gravel, and mud collected from subsurface seepage in localities that were dry at the surface. These were the only samples containing any free water.

b) *Moist* and c) *dry soil*. These samples were separated arbitrarily by their dampness. Dry soil exhibited no dampness, while moist soil could be slightly damp or very damp, but it never contained visible water. It is believed that all moist soil samples contained interstitial spaces with water-saturated air, while pore spaces of dry soil samples probably were less than water saturated.

d) *Leaf litter*. Moist leaf material was collected in May and June 1964 from former pools that had had no visible surface water for at least a week.

The numbers and kinds of organisms present at any time will depend in part on the season the samples were collected in relation to the life cycles of the separate species. Another important factor is the number and size of the interstitial spaces. Approximately 200 meters before Caldwell Hollow empties into Salt Creek, a dirt road fords it (Fig. 1). During periods of rain, water in the stream above the ford is always clear, whereas drainage from the road muddies the stream below the ford and fills interstitial spaces of the stream bed. Soil samples from the lower area contained no aquatic invertebrates at all except for one adult *Helichus basalis*. This is believed due directly to the lack of interstitial spaces in this area.

Moist leaf litter apparently serves as a final, temporary "oasis" for large aquatic animals remaining in the dried-up pools (Table 8). The crustaceans here were chiefly large adults, many in both species being ovigerous. They were too large to follow the subsiding water table. Coleoptera were sparse in leaf litter, but this was due in part to their temporal distribution. The only other group present were the caddisflies *Ironoquia* and *Lepidostoma*. Both are suspected of having aestivating "terrestrial-phase" larvae and pupae. The prepupal condition was observed in both species from moist leaf litter.

Animals living in moist soil or subsurface seepage must either be small enough to inhabit the existing interstitial spaces or else have the capability to actively burrow into the soil. In the latter group belong *Orconectes*, *Eurycea*, and possibly some of the adult beetles. The remaining aquatic animals are small enough to survive in the pore spaces *per se*.

The size of the interstitial spaces becomes increasingly important as the stream dries up and the water table subsides. As would be expected,

the large pore spaces are near the surface of the stream bed, and they decrease in size with depth. In May and early June the subsurface seepage was high in the stream bed, occupying relatively large pore spaces and maintaining water-saturated air in spaces immediately above it. Fairly large specimens of *L. fontinalis* (14 mm), *C. forbesi* (8 mm), and *I. punctatissima* (10 mm) were taken from the soil and seepage at this time. As the dry season progressed the seepage moved deeper into the stream bed, withdrawing from the larger interstitial spaces and hence making the survival of these large animals progressively more difficult. A parallel effect of this was that the water-saturated air, located above the water table, was also restricted to deeper levels containing smaller pore spaces. Short periods of rain temporarily raised the levels both of seepage and of spaces containing water-saturated air.

Organisms restricted to free water (subsurface seepage) or even those that can survive in moist soil must be of a small size during this time. During October 1963 *L. fontinalis* from moist soil averaged 3 mm in length, and in November 1963 they averaged 5 mm from subsurface seepage. Specimens of *C. forbesi* from moist soil averaged 3 mm in length during October 1963. Because of its obligate univoltine life cycle, all specimens of *C. forbesi* would be expected to be small in late summer and early autumn regardless of the stream's water characteristics. In this instance small interstitial spaces do not actively select against the large animals, of which there are none, but instead simply furnish a suitable space matrix that can be exploited by the species at a favorable time in its life cycle.

In contrast, *L. fontinalis* in permanent waters has large specimens throughout the year, which is probably its typical condition. In the dry stream, by late summer, the deep seepage and accompanying small spaces of water-saturated air are selective to small specimens of this species. As mentioned in an earlier section, the early mortality of some of the old generation, due to the above drought-related factors, accounts in part for its facultative univoltine cycle in Caldwell Hollow.

The occurrence of one large *L. fontinalis* (11 mm) in seepage during early August (Table 8) indicates that in rare instances in some locations, large interstitial spaces may extend to a considerable depth. Slack (1955), Paloumpis (1958), and Larimore *et al.* (1959), working with streams that regularly become interrupted in late summer, observed that isolated pools remaining through the dry period served as "havens" or reservoirs for aquatic animals. These pools, in many instances, were of prime importance in repopulating the streams when they again became continuous. Larimore *et al.* (1959), further pointed out that if the pools become stagnant, their inhabitants may be species unsuited for repopulation of the flowing stream.

In Caldwell Hollow the entire stream regularly becomes dry, and hence no pools can serve as repopulation foci. It is suggested that the nearest thing to a "stream haven" or faunal reservoir during the dry seasons is found in regions that contain relatively large interstitial spaces to a considerable depth. Those regions are very infrequent and almost always are located either immediately above or below former large pools, in areas where large quantities of loosely packed, coarse sandstone parti-

TABLE 8. The number and size range (mm) of species and groups of species from various types of soil samples. Samples have been grouped by month. See text for explanation of categories.

	Subsurface seepage			Moist soil			Moist leaf litter			Dry soil	
	Mo./yr.	No.	Size (mm)	Mo./yr.	No.	Size (mm)	Mo./yr.	No.	Size (mm)	Mo./yr.	No.
ISOPODA											
<i>Lirceus fontinalis</i>	Oct/63	1	9	Oct/63	26	2-8	May/64	75	3-14		
	Nov/63	21	3-9	May/64	11	2-6	Jun/64	120	3-10		
	May/64	14	2-14	Jun/64	25	3-7					
	Jul/64	4	7-9	Aug/64	2	8					
	Aug/64	3	4-11								
<i>Asellus stygius</i>	Oct/63	1	4								
	Jun/64	1	3								
	Jul/64	1	3								
	Aug/64	1	4								
AMPHIPODA											
<i>Crangonyx forbesi</i>	Nov/63	3	3-5	Oct/63	11	3-4	May/64	122	2-14		
	May/64	12	2-8	May/64	3	2-5					
	Aug/64	4	3	Jun/64	3						
DECAPODA	Nov/63	1	24								
<i>Orconectes propinquus</i>											
PLECOPTERA	Jun/64	7	2								
<i>Allocapnia pygmaea</i>											
EPHEMEROPTERA	May/64	2	4								
<i>Heptagenia</i> sp.											
ODONATA											
<i>Cordulegaster obliquus</i>				Oct/63	1	12					
TRICHOPTERA											
<i>Isonychia punctatissima</i>				Jun/64	1	10	May/64	2	15		
							Jun/64	2	9		
							May/64	4	5		
<i>Lepidostoma</i> sp.							Jun/64	4	3-4		

COLEOPTERA									
<i>Hydroporus laetus</i>									
	Oct/63	20	4	Oct/63	11	4			
	Nov/63	1	5*	Aug/64	26	4			
	Aug/64	1	4						
	Nov/64	11	4						
<i>Cymbiodyta</i> sp.									
	Oct/63	1	7	Aug/64	2	7			
	Nov/63	1	7						
<i>Helichus basalis</i>									
	Aug/64	2	5	Oct/63	2	5	May/64	1	5
				Aug/64	1	5	Jun/64	3	5
<i>Limnebius discolor</i>									
	Oct/63	18	2	Oct/63	6	2	Jun/64	1	2
				Aug/64	9	2			
DIPTERA									
<i>Tipulidae</i>									
	Jul/64	3	4-7	Oct/63	1	3			
				Aug/64	1	3			
<i>Tabanus</i> sp.									
				Oct/63	3	3			
<i>Tendipedidae</i>									
				Oct/63	1	3		Oct/63	1
									4
URODELA									
	Oct/63	1	18	Oct/63	7	16-20		Nov/64	1
									Adult
<i>Eurycea bislineata</i>									
NEMATOMORPHA									
<i>Gordius</i> sp.									
								Oct/63	4
									250-350
Number of each type soil sample collected by year and month									
1963									
Oct.		2			6			3	0
Nov.		3			0			4	0
1964									
May		2			1			0	2
June		0			1			1	1
July		2			0			1	0
Aug.		2			2			0	0
Sept.		0			1			0	0
Oct.		0			2			0	0
Nov.		0			2			1	0

* larvae

cles have accumulated. They are seldom located beneath the dry pool itself, because the pool, prior to its drying up, serves as a collecting basin for silt and small particles of detritus washed in by short periods of rain. These materials, in turn, settle into the interstitial spaces, reducing their size or eliminating them completely. Stagnation, as mentioned above by Larimore *et al.* (1959), also is characteristic of temporary pools formed during the dry periods in Caldwell Hollow. It restricts the inhabitants to adventitious species such as *Culex* and *Tubifera* and probably also affects adversely the interstitial fauna below the bed surface of the pools.

All species small enough to live in subsurface seepage do not necessarily survive in moist soil. The blind isopod *Asellus stygius* (Table 8) apparently is restricted to regions containing some subterranean water, as likewise are the small nymphs of *Allocapnia pygmaea*. In contrast, the two dominant crustaceans and adult aquatic beetles survive in spaces of water-saturated air above subsurface seepage as well as in the seepage itself.

Dry soil samples contained few organisms (Table 8). The physiological processes that maintained *Gordius* and the one tendipedid larva in dry soil are not known. Since the dry soil probably contained less than 100% relative humidity the organisms must be able to tolerate a certain fall in their moisture content. Hinton (1953) mentions that most insects can tolerate a fall in tissue moisture content of only 10 to 15 percent. He suggests those adapted to an environment that is periodically dry may take a longer time in losing this percentage. Hinton further points out, however, that a very few insects can survive much greater water losses. Among these are the larvae of the *Polypedilium vanderplankei* Hinton, found in shallow rock pools in Africa. At 65° C, in an electric oven, the larvae survived for 20 hours. In Caldwell Hollow the presence of aquatic animals, especially the dominant crustaceans, in moist soil and their absence in dry soil illustrates the narrow physical but wide ecological and physiological differences between soil pore spaces at 100% relative humidity and soil pore spaces even slightly less. This is exemplified by experiments of Edney (1951), which demonstrated that of seven species of terrestrial isopods none could gain weight and recover from desiccation at 95% relative humidity, only one species could at 98% relative humidity, while all recovered at 100% relative humidity.

DISCUSSION

With the data obtained in this study it is possible to discern certain important prerequisites for the maintenance of the permanent aquatic fauna in Caldwell Hollow. It is possible also to project and/or compare these essentials with other intermittent streams where biological studies have been carried out.

A semblance of the aquatic environment was present at all times in Caldwell Hollow. Even though the stream regularly "dried up" during the summer, water still persisted below the bed surface. This sufficed for the survival of an aquatic fauna, either in seepage itself or in water-saturated air spaces above the seepage. Survival in either locality is dependent on the availability of interstitial spaces, which in turn are determined

fundamentally by stream gradient and local geology. The stream bed of Caldwell Hollow was composed mainly of non-angular sandstone fragments, which in many places extended to a considerable depth. The particles promoted the formation of numerous and relatively large interstitial spaces. Besides its shading effect, the wooded, pristine watershed accounted for a low, almost nonexistent silt load, thus maintaining the pore spaces in a relatively unclogged state. It is likely that short periods of rain during the dry seasons also aid in maintaining pore spaces by washing out accumulated detrital materials from broad reaches of the stream bed and transporting them out of the stream entirely or depositing them in the limited areas of former pools.

Besides providing a habitable environment during the dry seasons (and in other seasons) interstitial spaces also effect the velocity of the water. Jaag and Ambühl (1964) experimentally demonstrated that close to the substratum the movement of water is slowed by friction, even to the extent of creating "dead-water" zones in fissures and pore spaces between stones. These areas where flow is strongly retarded are known as Prandtl's layers after their discoverer. Jaag and Ambühl (1964) report that rheophilic animals spend most of their lives in these small, relatively quiescent water spaces.

In certain geographical regions of North America, intermittent streams have stream beds comprised chiefly of exposed bed rock or with some other type of impervious layer close to the surface. Even short periods of heavy rain will flow over the land surfaces resulting in devastating flash floods. Such events are especially common in the southwestern United States (see Leopold and Miller, 1956; John, 1964). In such streams the few "dead-water" regions, which are located near the surface of the stream bed, can not be exploited by large numbers of animals. If the organisms are not strong enough to maintain themselves against the current, heavy mortality can ensue. This is possibly one of several reasons why many small intermittent streams of the southwestern United States support a very limited macroinvertebrate population, even though the stream may have a long spring flow period. In contrast, storm water in Caldwell Hollow, and over the Norman Upland in general, infiltrates into the stream bed with much subsurface movement. It takes exceedingly heavy rains to cause high water velocities, and even when this does occur it is suspected that the aquatic fauna can find protection in relatively deeper areas of the stream bed where the numerous interstitial spaces afford protection from strong water movements.

An interesting aspect associated with water velocities is that of the stability of the stream bed and its effect on the aquatic fauna, especially during periods of high water. Since most of the stream bed of Caldwell Hollow is composed of small, non-angular fragments, the stream would be expected to be rather unstable during maximum flow. Slack (1955) observed such instability for a larger stream also located in the Norman Upland. Kamler and Riedel (1960), studying an intermittent stream in Poland, presented time-series graph of its bottom configurations that would indicate a highly unstable bed during periods of even less than normal flow, although this phenomenon itself was not discussed.

Major changes occurred in the stream bed of Caldwell Hollow during the flood of March 1964. At this time, entire riffle areas were scoured out, in some places to depths of over 60 cm. Old pools were destroyed and new ones formed. These events must have caused considerable mortality of animals inhabiting interstitial spaces. This was the only time during the study period that the stream channel was filled from bank to bank and even for a short period overflowed its banks. It was also the only time that obvious changes in the stream bed were noted. Nevertheless, minor disturbances undoubtedly occurred whenever the stream was continuous. Instability would be confined to that part of the channel with high water velocities, *i.e.*, surface flow. It would not affect appreciably the stability of regions with only subsurface seepage, which in many localities can occupy more of the stream channel than surface flow itself. In short the instability affects only a part of the habitable stream bed, and even here for only short periods.

A final aspect relating to interstitial spaces is that of the "freeze-thaw" characteristics of small streams. Newsom (as reported by Malott, 1922, p. 175) mentions that strata in the Norman Upland are soft, absorb water easily, and therefore are easily fractured by freezing. Streams entering Salt Creek from the north side, such as Caldwell Hollow, are protected from wide temperature variations, especially in the winter. In contrast streams entering from the south are exposed to wider winter temperature fluctuations, freezing at night and thawing during the day. The results are muddy, silt-laden streams flowing from the south and clear, or frozen streams entering from the north. The adverse effect of silt on interstitial spaces has been mentioned previously and would seem to be especially prevalent in intermittent streams entering Salt Creek from the south. A few spot samples taken from one of these streams in May 1964 yielded few macroinvertebrates and would tend to support such an assumption.

Turning to the permanent aquatic fauna of Caldwell Hollow, small size was a necessity for most species during the dry seasons. With the exception of the apparently highly adapted caddisflies, the permanent fauna survived in small pore spaces. Specimens of *Hydroporus laetus*, *L. fontinalis*, and *C. forbesi* were found as deep as 60 cm, although most were found between the surface and 25 cm. Small specimens of *C. forbesi* appeared to penetrate to the greatest depth. The consequences of the dry seasons on the species size has been discussed previously. The permanent macroinvertebrate fauna is restricted mainly to animals with a univoltine spring cycle, with a life span of not more than a year, or without a major growth period in summer or an autumn emergence.

Finally, it would be convenient to have the proper perspective of the intermittent nature of Caldwell Hollow in regards to other intermittent streams where biological studies have been carried out. No single hydrological or climatological parameter will suffice to classify intermittency, at least to the satisfaction of biologists. In the broadest sense natural aquatic channels are usually described by hydrologists as ephemeral, intermittent, or perennial (or permanent) in respect to their flow characteristics, *i.e.*, an ephemeral stream carries water only during storms, an intermittent stream is one where dry stretches alternate with flowing or standing

water areas at low flow, and a perennial stream carries some flow at all times. Perennial rivers and ephemeral rills are easily separated, but the gradation from perennial streams to intermittent or especially from intermittent to ephemeral stream is often difficult to resolve objectively. For example Caldwell Hollow carries visible water for periods much longer than could result from a single storm, but at "low flow" the stream bed is completely dry.

Usinger (1956, p. 15) presents a classification of intermittent streams of California from a scheme originally formulated by Dana L. Abell (personal communication: Usinger, 1965), in which the streams are grouped by their temporal flow characteristics—*i.e.*, short flow, long flow, etc. For example, the intermittent foothill stream of California having an eight-month flow period, in which Abell (1959) studied a mosquito population, would be described as a long-flow, fluctuating, intermittent stream. Still, from a biological point of view, many local features must be considered, especially during the dry season. Of paramount importance are such components as the nature of the stream bed, water table characteristics, and the shading effect. These elements in turn depend on such a variety of geographical, geological, and climatological factors that it is beyond the scope of this report to discuss them critically in any depth.

To elaborate briefly, an intermittent stream with a long-flow period may be reduced only to isolated pools during the regularly occurring dry period. In this case, it may support a permanent and varied fauna, even a substantial fish population. In contrast, if the stream becomes completely dry during the non-flow period but retains relatively high subsurface seepage with large interstitial spaces, it may support a permanent aquatic fauna, but restricted as regards life cycles (*e.g.*, univoltine, annual, etc.)—somewhat similar to Caldwell Hollow, which, however, has a short-flow period. In still another situation the stream regularly may go completely dry during the non-flow period, with subsurface seepage drastically reduced or nonexistent. Paralleling this, the stream may exhibit an impervious stream bed having few interstitial spaces, or, as found in some arid western regions, the watershed may not be wooded, resulting in high stream-bed temperatures. In this instance, the stream's fauna may not be permanent but limited instead to adventitious and migratory species, a situation similar in many respects to that of an ephemeral rill. Although the forementioned are only idealized situations, the point is that the permanency, variety, and adaptability of aquatic fauna in so-called intermittent streams depend, among other things, on many subtle physical components. Such components are not necessarily reflected by a few criteria, descriptive of the stream's flow and non-flow state.

With the above in mind, a comparison was made between Caldwell Hollow and several other intermittent streams where pertinent biological information is available. The arrangement given below relies not so much on the described flow characteristics of the stream as on the permanency of its aquatic environment. Permanency in turn was assumed to be reflected by the type of aquatic animals (especially the life cycles of the macroinvertebrates) living in the stream. The supposition is that the dry period usually occurs in summer (at least in temperate regions), and,

depending on the physical nature of the individual stream, tends to eliminate the following animals: 1) those with major growth during the dry season, including non-annual animals, *i.e.*, those with a life span of two years or more; 2) insects with a late summer or fall emergence; 3) macroinvertebrates with bi- or multivoltine reproductive characteristics, *e.g.*, those producing a generation in the fall (as well as in the spring). In short, the fauna of a stream may be a better overall reflection of the intermittent nature of the stream than are the few described parameters relating to its flowing or non-flowing period. It should be pointed out in the following arrangement that the amount of faunistic information varied widely from stream to stream, and in some instances the life cycle of a particular invertebrate could be inferred only from data of other studies. The River Susaa was used only as a reference point and because extensive faunistic information is available for it. The streams are arranged in decreasing order of permanency of their aquatic environment.

1. Permanent stream. **River Susaa, Denmark** (Berg, 1948).
 Fish: several families, including the large carnivorous species *Salmo trutta* L. and *Esox lucius* L.
 Invertebrates: includes species with possible bivoltine cycles (*e.g.*, *Caenis*); with two-or-more-year life span (*e.g.*, *Aeschna*); with fall emergence (*e.g.*, *Centroptilum*).
2. Drought-stricken (Aug.—Oct.) permanent stream. **Afont Hirnant, Wales** (Hynes, 1958).
 Fish: *Salmo trutta* and *Cottus*.
 Invertebrates: contains species with life cycles similar to those of River Susaa but temporary elimination in affected area of fall-emerging insects (*e.g.*, *Ephemerella ignita*) and of those with a bivoltine cycle or with major summer growth (*e.g.*, *Baetis tenax*, *Diura bicaudata*, *Perlodes microcephala*). After the occurrence of another egg-laying period, the above species reappeared in the former drought area, being colonized from unaffected reaches of the stream.
3. Intermittent stream. **Swinski stream, Poland** (Kamler and Riedel, 1960).
 Fish: no information available.
 Invertebrates: contains several bivoltine species and species with a major growth period in summer (*e.g.*, *Baetis* spp., *Chaetopteryx villosa*, *Ecdyonurus venosus*).
4. Intermittent stream. **Smiths Branch, Illinois** (Larimore *et al.*, 1959).
 Fish: several families (*e.g.*, centrachids, cyprinids, catostomids).
 Invertebrates: contains a few with bivoltine cycles or with fall emergence (*e.g.*, *Caenis*, *Ephoron*, *Cheumatopsyche*), and a few with life spans of possibly two years or more (*e.g.*, *Acroneuria*, *Corydalis cornutus*). The fauna was studied most intensely during a severe drought that extended over a period of years and dried up reaches that are normally wet even during the regularly occurring dry season. The drought temporarily eliminated the above species, although univoltine

species with spring emergence survived (e.g., *Allocapnia*, *Rhyacophila lobifer*). Other invertebrates also surviving included adult aquatic beetles, tendipedids, and the isopod *Asellus*, probably *brevicaudus* Forbes.

5. Intermittent stream. **Brummets Creek, Indiana** (Slack, 1955).

Fish: similar to Smiths Branch.

Invertebrates: Although for the most part, the fauna was not broken down lower than order, summer and fall emerging *Hexagenia* were reported. Personal observation indicated bivoltine and two-year-life-span species present also.

6. Intermittent stream. **Rock Riffle, Ohio** (Stehr and Branson, 1938).

Fish: limited to minnows (e.g., *Notropis cornutus*), which are present only in fall, winter, and spring in lower reaches.

Invertebrates: contained a few with possible major growth period in summer (e.g., hydropsychids) or with a two-or-more-year life span (e.g., *Corydalis cornutus*). Much of the fauna was restricted to univoltine species with spring or winter emergence (e.g., Capniidae).

7. Intermittent stream. **Caldwell Hollow, Indiana**.

Fish: limited to a temporary population of minnows (e.g., *Semotilus atromaculatus*), present only during the spring months.

Invertebrates: restricted to univoltine species without a major summer or early fall growth period. With the exception of a few Coleoptera, all species have an annual cycle. Furthermore, with the exception of some highly adapted caddisflies, all insects emerge in spring.

Several other important studies have not been arranged in the above sequence because they were directed specifically to one group of aquatic animals. These include Abell's (1959) investigation of the mosquito population of a California intermittent stream, Paloumpis' (1958) study of fish in an Iowa intermittent stream, and John's (1954) study of fish in intermittent streams of Arizona.

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Coexistence in *Chaoborus* and its Ecological Significance

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ABSTBACT

The number of *Chaoborus* species was compared in 59 lakes in North America, South America, Europe, and Africa. Two or more species coexisted in 45 of these lakes on all four continents. Probably all sympatric species can coexist. Of the 14 lakes having only one species, 11 were in Europe, 3 in North America. In Europe *C. flavicans* coexisted with other sympatric species in ponds, but was the only species living in lakes deeper than 5 m. It can withstand hydrostatic pressure as great as 4 atmospheres, while the sympatric species, *C. crystallinus*, *C. obscuripes*, and *C. pallidus*, apparently cannot withstand more than 0.5 atmosphere of hydrostatic pressure. In two of the North American one-species lakes no other species lives in the area. The third lake may have been inadequately sampled.

The commonness and pattern of coexistence indicate that competitive exclusion does not occur in natural populations of *Chaoborus*. It is suggested that coexisting species of *Chaoborus* do not compete because the populations are below the level at which a shortage of common resources occurs.

TABLE OF CONTENTS

	Page
Introduction	99
Identification of species	100
Ecology of <i>Chaoborus</i>	102
Frequency and distribution of populations of coexisting species of <i>Chaoborus</i>	103
Lake categories in which <i>Chaoborus</i> species live	107
Discussion	110
Acknowledgments	111
References	111

INTRODUCTION

Two species of *Chaoborus* were found in the same lake as early as 1937 by Berg. Since then there have been several scattered records of other lakes with two or more species (Main, 1953; Cook, 1956; MacDonald, 1956; Wood, 1956; James and Smith, 1958; Stahl, 1959; Hirvenoja, 1960, 1961; and Judd, 1960, 1961, 1964), but no one has considered the ecological significance of such coexistence. It seemed to me that such populations might be more common than had been realized heretofore, and that study of them might help elucidate the interactions between closely related species living in the same place. Many instances of coexistence of related

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species have been studied. Much debate continues on the importance of interspecific competition and competitive exclusion in determining the distribution and abundance of animals. Therefore, more research is needed (Mayr, 1963).

A study of coexistence in *Chaoborus* was especially attractive for four reasons: the taxonomy of the genus is simple, the larvae can usually be identified to species with accuracy and facility, they are common in lakes and ponds all over the world, and they may live in a relatively uniform habitat.

The aims of this investigation, then, were to find out how common coexistence of species is in *Chaoborus*, what patterns there are to the coexistence, and why coexistence does or does not occur in a given lake. To achieve these aims larvae were identified from a number of lakes, and published records were evaluated.

IDENTIFICATION OF SPECIES

Accurate identification is essential for determining the ecological characteristics of a species. In *Chaoborus*, larvae of most species are more easily identified than adults, and the larvae can be collected at any time of year. Therefore, the larvae have been used almost exclusively in this investigation. Because the use of larvae as records of species is basic to the present investigation and because some misidentifications exist in the literature, the pertinent taxonomic references are considered briefly.

For North America Cook (1956) is the basic reference. Cook found eight Nearctic species of *Chaoborus*. He described the larvae of all but one, *C. annulatus*, which occurs only in Florida and Georgia. The larvae of *C. punctipennis* and *C. astictopus* are apparently identical, but the former is found east of the Rocky Mountains, the latter west of them. *C. albatus* is distinguished from *C. punctipennis* only by the broader prelabral appendages; some borderline cases arise. Additional information on the immature stages of *C. borealis* was provided by Smith (1960a), who also (1960b) compared certain measurements of *C. americanus*, *C. borealis*, and *C. nyblaei*. A new species, *C. maculipes*, was described by Stone (1965), but the larva is as yet unknown. This species is known only from Arkansas and Louisiana.

Peus (1934) is the basic reference for Europe. Peus described the larvae of the four most widespread European species, *flavicans*, *crystallinus*, *obscuripes*, and *pallidus*. Specimens of *C. nyblaei*, which lives only in northern Europe, were not available to him. Additional descriptions of *flavicans* and *crystallinus* were given by Berg (1937). Prokešová (1959) presented a key to all instars of the four species described by Peus (1934). In 1938 Peus described a new species, *C. alpinus*, that is very similar to *flavicans*. A key to the mature larvae, pupae, and adults of all known European species, including *alpinus* and *nyblaei*, was presented by Hirvenoja (1961). This paper contains the first description of European specimens of *nyblaei*.

It is questionable whether *alpinus* is a valid species. At all stages of its life history this species is very similar to *flavicans*. The larvae are not

distinguishable. The pupae differ only slightly in the shape of the respiratory horns. Male adults of the two species are distinguished only by a minor difference in their penis valves (= genital sclerites). Concerning the variation in the penis valves of *flavicans* Cook (1956, p. 23) says, "The variation in the penis valves of the males is quite marked, so that if only the extremes were available it might seem that two distinct species are involved (figure 16H). However, with an adequate sample of specimens all intergrades can be found, and it becomes impossible to segregate individual specimens. Even this might not invalidate the possibility that two or more species are involved except that both extremes and some intergrades have been found in single homogeneous samples. Single specimens have been seen in which the penis valves differed widely from each other." In view of Cook's observations, and also because *alpinus* and *flavicans* seem to have virtually identical ecological characteristics (see below), it seems best to call *alpinus* a synonym of *flavicans*.

A related question is whether *C. flavicans* is the same species in North America and in Europe. According to M. Hirvenoja (pers. comm.), Mr. Ole-A. Sæther has found one difference in the chaetotaxy of the larvae, and one in the pupae. Possibly these differences are sufficient to indicate specific distinction, but in my opinion the information available favors the view that they are not and that *C. flavicans* is a truly Holarctic species.

It has been a common practice in many European studies (especially the older ones) to call any *Chaoborus* collected from a lake *crystallinus* (or its synonym, *plumicornis*). This practice may have stemmed in part from the statements by Peus (1934, pp. 642, 661) that *crystallinus* lives at depths as great as 40 m. Evidently *flavicans* can live at such depths, but *crystallinus* can not. Peus based his statements on Wesenberg-Lund (1914), who found the "lake form" of *plumicornis* at depths as great as 40 m. From Wesenberg-Lund's drawing of the prelabral appendage of the lake form, Peus assumed the species was *crystallinus*, although he overlooked the fact that *flavicans* has the same shape of prelabral appendage. The mandibles distinguish the two species, but Wesenberg-Lund did not present drawings of these. Therefore, on the basis of the evidence he presented, his "lake form" could just as easily have been *flavicans*. Indeed, Berg (1937, p. 10) has already pointed out that in Esrom Lake, which was one of the lakes sampled by Wesenberg-Lund, the species present is *flavicans*.

Reports of *crystallinus* from lakes deeper than 5 m include those by Humphries (1936), Lundbeck (1936), Thomas (1944), Findenegg (1955), Frey (1955), and Kajak (1961). I examined larvae from Windermere, England, the lake studied by Humphries, and found only *flavicans* (Table 3). An examination of Frey's camera lucida drawings of two mandibles from the sediments of Längsee, Austria, showed that they, too, are almost certainly *flavicans*. None of the other records of *crystallinus* presented above is supported by drawings or other evidence that the species was accurately identified. In every instance in which identification of *crystallinus* is certain, the species has been found only in shallow waters, no deeper than 5 m.

Taxonomic information on the species of *Chaoborus* in South America and Africa is sparse. The names of Species 1 and Species 2 from Brazil

are not known, but there is no doubt that two species are represented. For Ekunu Bay in Lake Victoria, MacDonald (1956) was able to identify reared adults of his species B only to the subgenus *Sayomyia*. *C. (Sayomyia) pallidipes* is known from other parts of this lake, but none of MacDonald's reared adults was of this species.

In many studies of the benthos no attempt has been made to identify *Chaoborus* beyond genus, and even in those in which an identification to species was made, apparently only a few specimens were examined. Because such meager data do not prove the absence of a second species, these records cannot be used in the present study. For example, in three lakes in which one species (or two) had been reported by previous investigators, I found an additional species. These are Sugarloaf Lake, Costello Lake, and Little McCauley Lake, studied by Beatty and Hooper (1958), Miller (1941), and Wood (1956), respectively. The criteria for including a lake in the present study were (1) the confirmed presence of two or more species, or (2) at least 100 identified specimens of a single species, in which case the lake was considered as having only this one species. Published records that did not list the number of specimens examined were included as instances of one-species population if I judged (or was told) that the investigator had examined a sufficient number of specimens.

ECOLOGY OF *Chaoborus*

Chaoborus larvae may be abundant in ponds and also in large lakes. In the latter the larvae are typically restricted to deep water. During the day the larvae in such lakes live in the mud of the profundal zone. At night they migrate into the surface waters, and it is here that probably all feeding is done, copepods being the favored food (Berg, 1937; Main, 1953). Young larvae tend to be planktonic at all times, although they are found in the deeper water during the day, and migrate nearer to the surface at night. The larvae are abundant in those lakes whose lower waters become depleted in oxygen (Thienemann, 1922; Findenegg, 1955). For the lower waters of a lake to become depleted in oxygen, the lake must be stratified sufficiently that the upper waters do not mix with the lower waters.

Because of the importance of stratification in influencing the environmental conditions likely to be significant for *Chaoborus*, a classification of lakes based on stratification is useful in considering the ecology of *Chaoborus*. Lakes that are sufficiently deep or protected from wind action become thermally stratified each summer into a warm, well-mixed epilimnion, and a cold, stagnant hypolimnion. Holomictic lakes are those that become mixed one or more times each year, typically each spring and autumn. They contrast with meromictic lakes in which part of the deep water (the monimolimnion) is stabilized by dissolved substances rather than by low temperature, and therefore does not undergo seasonal mixing. Stratification tends to be permanent in such lakes. Holomictic lakes are subdivided into three categories: First-class lakes become stratified, and the temperature of the deep water does not rise appreciably above 4°C; second-class lakes become stratified, but the temperature of the hypolimnion does rise appreciably above 4°C; third-class lakes do not stratify. These lake categories are discussed in Hutchinson (1957, p. 440).

The development of stratification depends principally on area and depth, and on the relationship between these two parameters. For example, although the maximum depths of Lake Texoma and Oliver Lake are almost the same, the former is not stratified whereas the latter is because of the great difference in their areas (Table 3). For the purpose of this paper it seems desirable to place ponds, which are small third-class lakes, in a separate category. Pools and puddles, even if temporary, are called ponds. It seems better not to specify an area for "small." Thus the boundary between ponds and other third-class lakes is not sharp, but the extremes are readily distinguished.

The amount of oxygen depletion in the hypolimnion during stratification depends on morphometry, productivity, and duration of stratification. Large, deep lakes generally have little oxygen depletion. The tolerance of *Chaoborus* larvae to low oxygen concentrations explains their presence and abundance in oxygen-poor habitats, but it does not explain their scarcity in the littoral zone, or in the hypolimnion of lakes having little oxygen depletion. Juday (1921) suggested that the larvae use the anaerobic hypolimnion as a refuge from predators, which receives support from the findings of Jónasson (1965) that mortality of *Chironomus anthracinus* from predation is low during stratification in Esrom Lake.

The meager and scattered evidence available suggests that other factors, such as pH, color of water, and alkalinity, do not have any significant effect on the distribution and abundance of *Chaoborus* larvae.

The above description of the ecology of *Chaoborus* is true for the genus, but is not necessarily true for all species in the genus. However, a study of the two coexisting species in Myers Lake, Indiana, *flavicans* and *punctipennis*, shows that these two species have a similar distribution within the lake, and have similar niches (Stahl, 1966). If this is true for these two species in Myers Lake, it seems reasonable to assume that it is true for these two species in other lakes, and that it is probably true for other combinations of species. Thus, it is assumed that in each instance of co-occurrence the species are living in the same habitat within the lake and have essentially the same ecological niche.

FREQUENCY AND DISTRIBUTION OF POPULATIONS OF COEXISTING SPECIES OF *Chaoborus*

Of the 59 lakes that met the criteria for inclusion in this study (Tables 1, 2, and 3), three-fourths of them (45) have more than one species. Lakes with two or more species have been found in North America, South America, Europe, and Africa. Lakes with only one species are much more restricted in their distribution; of the 14 such lakes, 11 are in Europe, and 3 are in North America. Thus coexistence is the usual condition in *Chaoborus*, and of the one-species populations almost all are restricted to one area.

All the seven Nearctic species whose larval stage is known (thus excluding *annulatus* and *maculipes*) have been found coexisting with at least one other species (Table 1). Of the 21 possible pairs that could be formed by 7 species, 4 pairs can definitely be excluded, because the species are not sympatric (Cook, 1956). Of the remaining 17 pairs, 10 have been

TABLE 1. Lakes in North America with more than one species of *Chaoborus*. Lake categories are: II = second-class lake, III = third-class lake, P = pond, M = meromictic lake (see text for further explanation). Lake dimensions in parentheses are estimates. Unless otherwise indicated, all identifications and counts were made by the author or by W. P. Coffman.

Species/Lake	No. of larvae	Species in order listed			Lake category	Area (ha)	Max. depth (m)
		1 %	2 %	3 %			
<hr/>							
<i>borealis-americanus</i>							
Pool near Churchill, Manitoba (James and Smith, 1958)					P		<1
<i>flavicans-americanus</i>							
Kepple, Washington	169	2	98		III	32	2.5
Corbett, British Columbia	90	97	3		II	20	19
<i>flavicans-astictopus</i>							
Knott's Pond, Washington	28	71	29		III	0.06	0.7
Siltcoos, Oregon (Cook, 1956)					II ?		
<i>flavicans-nyblaei</i>							
Hall, Washington (Main, 1953)	183	10	90		M	2.8	16
<i>flavicans-punctipennis</i>							
Costello, Ontario	246	8	92		II	38	20
Winona, Indiana	418	9	91		II	215	24.4
Myers, Indiana	4126	12	88		II	39	17.9
South Walker, Ontario (Judd, 1960)	624	12	88		II	(35)	8
Spear, Indiana	207	26	74		II	16	8
Saunders, Ontario (Judd, 1964)	4134	27	73		II	(35)	9
Pretty, Indiana	13	34	66		II	75	25
Pog, Ontario	7	43	57		II	(30)	(11)
Redmond's, Ontario (Judd, 1961)	2775	97	3		P	(0.3)	1.5
<i>albatus-punctipennis</i>							
Maxinkuckee, Indiana	26	8	92		II	751	26.5
Wylie, Indiana	79	8	92		II	(3)	(4.5)
Sugarloaf, Michigan	155	56	44		III	73	6
Wawasee, Indiana	5	60	40		II	1200	23.5
<i>flavicans-punctipennis-albatus</i>							
Tippecanoe, Indiana	158	<1*	96	3	II	286	37.5
Wyland, Indiana	635	<1	95	5	II	3	6
Indian Village, Indiana	46	6	85	9	II	2	6.5
Little McCauley, Ontario	258	14	80	6	II	20	10
Manitou, Indiana	31	51	26	23	II	289	14.9
<i>flavicans-punctipennis-nyblaei</i>							
Tub, Wisconsin	338	6	93	<1*	II	0.8	8.0
Helmet, Wisconsin	17	41	41	18	II	3.0	10.4
Mud, Wisconsin	55	62	22	16	II	5.5	15.7
<i>flavicans-punctipennis-nyblaei-americanus</i>							
Stewart's Dark, Wisconsin	222	2	39	3 56	M	0.7	8.8

* Only one specimen.

found. This is a high enough proportion to suggest that all species living in the same area can be expected to coexist in each lake or pond suitable for *Chaoborus*. The two most widespread and common species, *flavicans* and *punctipennis*, are also the species involved in the most pairs. Four of the pairs not found involve *borealis*, for which only one record is available.

If it is true that all sympatric species can co-occur, then in every area in which three (or four) species live, each lake suitable for *Chaoborus* should be expected to have these three (or four) species coexisting in it. To demonstrate that no more than two species occur in a lake it is necessary to examine a sufficiently large number of specimens, just as it is necessary to do this if the presence of only one species is to be established. Although such a criterion was not applied to the two-species populations included in Table 1, a number of records seem to include a sufficient number of identified specimens for examining this hypothesis.

The three areas in North America that are known to contain more than two species are: Indiana, Algonquin Park in northeastern Ontario, and Wisconsin. *C. flavicans* and *punctipennis* occur in all three areas, *albatus* occurs in Indiana and Algonquin Park, while *nyblaei* and *americanus* occur in Wisconsin. (The larval records of *nyblaei* should be checked by identification of adults, as Cook, 1956, specifically excludes Wisconsin from the range of this species.)

Of the 11 lakes in Indiana included in Table 1, 4 contain all three species known from the region, 4 have only two species based on fewer than 100 specimens identified, and 3 more have only two species but based on more than 200 specimens identified. Conceivably, even the 418 specimens identified from Winona Lake are not enough to prove the absence of *albatus*. There should be little question that the 4,126 larvae identified from Myers Lake are sufficient. Of these, only 4 were evidently *albatus*. Identification is provisional, because the one characteristic used to distinguish *albatus* from *punctipennis* is the width of the prelabral appendage, and this is subject to some variation in each species. Thus if these four larvae are *albatus* for certain, this species is present in such a low proportion (0.1%) that it is all but absent.

Of the lakes from Algonquin Park, Ontario—Little McCauley, Costello, and Pog—the three species known from the region have been found only in Little McCauley Lake. Certainly the 7 specimens examined from Pog Lake do not prove the absence of *albatus*, and perhaps the 246 specimens from Costello Lake are also insufficient to prove the absence of this species. (South Walker Pond, Redmond's Pond, and Saunders Pond are located in western Ontario, about 400 km west of Algonquin Park. *C. flavicans* and *punctipennis* are the only two species recovered from these lakes despite thorough sampling and thus are presumably the only species in that area.)

Three species have been found in all 4 of the Wisconsin lakes listed in Table 1, and a fourth species, *americanus*, in Stewart's Dark Lake. In view of the rather modest numbers counted it seems reasonable to assume that *americanus* probably occurs in the other three lakes also.

Thus it seems likely that in North America all species living in the same area can coexist, although some species may form a very small proportion of the total population.

In North America, populations of co-occurring species exist in all four categories of lakes.

Four of the five Palaearctic species are known to coexist in various combinations (Table 2). The exception is *pallidus*, which, although it has been reported from a number of ponds, has not been included in Table 2 or Table 3, because none of the records of it met the criteria for inclusion in those tables. Of the 10 pairs that could be formed by five species, 5 have been found. Four of the absent pairs are with *pallidus*, and the remaining pair, *flavicans-nyblaei*, is lacking, because these two species are not sympatric so far as is known. Thus in Europe also the evidence suggests that all sympatric species can coexist.

It is significant that seven of the eight lakes in Europe having more than one species are ponds, the one exception being a second-class lake near Yerseke only 5 m deep.

In the Amazon region of Brazil coexistence is the usual pattern (Table 2). No information is available on the lake category, area, or

TABLE 2. Lakes in Europe, Brazil, and Africa with more than one species of *Chaoborus*. Lake categories as in Table 1.

Species/Lake	No. of larvae	Species in order listed		Lake category	Area (ha)	Max. depth (m)
		1 (%)	2 (%)			
<i>flavicans-crystallinus</i>						
Sorte Dam, Denmark (Berg, 1937)	22	77	23	P	0.7	1
Lake near Yerseke, Netherlands (Parma, pers. Comm.)	15	60	40	II	0.01	5
Pool T ₂ , near 62°N, Finland (Hirvenoja, 1960)				P	0.09	0.5
Pool M ₂ , near 62°N, Finland (Hirvenoja, 1960)				P	0.2	0.2
<i>flavicans-obscuripes</i>						
Pool T ₁ , near 62°N, Finland (Hirvenoja, 1960)		<i>flavicans</i> dominant		P	0.24	2
<i>flavicans-crystallinus-obscuripes</i>						
Pool near The Hague, Netherlands (Parma, pers. comm.)		<i>crystallinus</i> dominant		P	0.009	0.5
Pool M ₁ , near 62°N, Finland (Hirvenoja, 1960)		<i>crystallinus</i> dominant		P	0.2	0.5
<i>nyblaei-crystallinus-obscuripes</i>						
Pond near 70°N, Finland (Hirvenoja, 1961)		<i>nyblaei</i> dominant		P	0.3	
species 1-species 2 (Brazil)						
Rio Maués-assú	9	22	78			11
Lago Curí	7	29	71			4
Lago Timbó	7	29	71			3.8
Lago S. Antonio do Iça	17	65	35			3.2
Rio Abacaxís	3	67	33			8
Lago do Rei	214	76	24			5.5
Lago Janauarí	95	97	3			4.5
Lago Salfado, Cab. do Boi	79	99	1*			4
<i>anomalous-species B</i>						
Victoria (Ekunu Bay), Africa (MacDonald, 1956)				III	68,800km ²	79

* Only one specimen available

maximum depth of these lakes. The depths listed are those at which each collection was made. The one record from Africa indicates that coexistence occurs on this continent. Larvae live to a depth of 30 m or so in Lake Victoria.

Lake Texoma and Clear Lake, California, two of the North American lakes that have only one species (Table 3), are in areas that apparently lack other species of *Chaoborus* (although *flavicans* and *nyblaei* might be sympatric with *astictopus* in the vicinity of Clear Lake). The presence of only *punctipennis* in Oliver Lake is puzzling, since all the other lakes in the area have *flavicans* and/or *albatus* as well. Perhaps in this instance even the 267 *punctipennis* larvae identified were inadequate to prove the absence of the other two species. It is probable that low numbers of *flavicans* and *albatus* exist in the lake; further study of Oliver Lake would be desirable to substantiate this.

In every one of the 11 European lakes having only one species, that species is *flavicans* (Table 3). Yet this same species coexists with other species in ponds in the same area (Table 2). None of the lakes in which *flavicans* occurs alone is a pond, and all but 3 are second-class lakes. Only two of the lakes are significantly shallower than 5 m.

LAKE CATEGORIES IN WHICH THE *Chaoborus* SPECIES LIVE

In North America all species of *Chaoborus* (except *annulatus* and *maculipes*) have been found in ponds (Table 4). Three species—*flavicans*, *punctipennis*, and *americanus*—have been found in all four lake categories, and all the remaining species except *borealis* have been found in three

TABLE 3. Lakes probably with only one species of *Chaoborus*. Lake categories as in Table 1.

Species/Lake	No. of larvae	Lake category	Area (ha)	Max. depth (m)
<i>punctipennis</i>				
Oliver, Indiana	267	II	145	28.5
Texoma, Oklahoma (Sublette, 1957)		III	38,320	29
<i>astictopus</i>				
Clear, California (Deonier, 1943)		III	16,190	14
<i>flavicans</i>				
Lake near Baarn, Netherlands (Parma, pers. comm.)		II	15	3
Frederiksborg, Denmark (Berg, 1937)		III		3.5
Grimsgöl, Sweden (Brundin, 1949)		II	1	4.8
Kempton Park East, England (Mundie, 1957)		III	16	7
Lake near Utrecht, Netherlands (Parma, pers. comm.)		II	4.7	12
Skärshultsjön, Sweden (Brundin, 1949)		II	36	14
Esthwaite Water, England (Mundie, pers. comm.)		II	100	16
Esrom, Denmark (Berg, 1937)		II	1,730	22
Lago di Varese, Italy (Bonomi, 1962)		II	1,495	25.7
Krottensee, Austria	139	M	9	47
Windermere, England	132	II	1,490	67

categories. It seems probable that with more collecting, all species except *borealis* would be found in all four lake categories. Possibly *borealis* is restricted to ponds. The statement by Cook (1956) that *punctipennis* and *astictopus* are the only Nearctic species occurring in large lakes is therefore incorrect.

In Europe the species differ strikingly in the lake categories in which they occur (Table 5). All species have been reported from ponds, but only *flavicans* has been found in all four lake categories. Quite likely *nyblaei* would be found to occur in other lake categories in Europe if more than one record were available, since it occurs in at least three lake categories in North America (Table 4). This assumes that it is a truly Holarctic species.

With the identification of *crystallinus* clarified, this species is seen to be restricted almost entirely to ponds. The one valid exception is the lake near Yerseke in the Netherlands, which is an unusually small second-class lake. Even though it is only 5 m deep its small area permits the development of thermal stratification. The finding of *crystallinus* as well as *flavicans* in this lake suggests that *crystallinus* can tolerate low oxygen concentrations as well as *flavicans*. The data in Table 5 suggest that *crystallinus*, *obscuripes*, and *pallidus* rarely or never occur in second-class, large third-class, and meromictic lakes because they are unable to withstand the hydrostatic pressure at depths greater than 5 m.

TABLE 4. Lake categories in which the North American species of *Chaoborus* have been found. Lake categories as in Table 1. Numerals refer to the number of lakes listed in Tables 1 and 3 in each category. Superscript numerals refer to supplementary information in the footnotes.

Species	Lake category			
	II	III	P	M
<i>albatus</i>	8	1 ¹	2	
<i>americanus</i>	1	1	1 ³	1
<i>astictopus</i>	1?	1 ⁴	1	
<i>borealis</i>			1 ⁵	
<i>flavicans</i>	18	1	1 ⁶	2
<i>nyblaei</i>	3		7	2
<i>punctipennis</i>	20	2	1 ⁸	1

¹ Found in Geiger Lake, Mississippi, by Woodsmansee and Grantham (1961).
² Found in several pools by Haufe (1952).
³ Jenkins and Knight (1950) found this species in rock pools with plants.
⁴ Cook (1956) reported this species from several California and Oregon lakes, but no information on their category is available.
⁵ James and Smith (1958) found this species in several pools and ponds.
⁶ Cook (1956) reported larvae from "water-filled road ruts, temporary ponds, and small pools" but did not mention lakes. I collected one larva from a pond near Winona Lake, Indiana.
⁷ Jenkins and Knight (1950) and James and Smith (1958) found this species in several Canadian pools; larvae also collected in a shallow pond in northern Alaska by John Hobbie.
⁸ I found this species in two small ponds near Winona Lake, Indiana; Cook (1956) said they are found in small ponds as well as large lakes.

Wesenberg-Lund (1943, p. 416 ff.) discusses the effect of hydrostatic pressure on *Chaoborus* larvae, based on his own observations and those of Krogh, Bardenfleth, and Ege. The larvae Krogh used in his laboratory experiments died in a few days when kept under one extra atmosphere of pressure. Yet Wesenberg-Lund collected larvae at depths of 40 m, where they were living at four atmospheres of excess pressure. Moreover, the lake larvae withstood a quick change to normal pressure without any difficulty. To help clarify these conflicting observations Bardenfleth measured the thickness of the walls of the tracheal air sacs of "lake larvae" and "pond larvae." In the lake larvae the walls were twice as thick as in the pond larvae. Ege established that three times as much pressure was needed to compress the sacs of lake larvae a given amount as to compress the sacs of pond larvae, suggesting that larvae can tolerate only a limited amount of compression of the air sacs. None of these investigators identified their larvae to species, but judging from the data in Table 5 (this paper) the lake larvae were *flavicans* and the pond larvae were *crystallinus*, *obscuripes*, or *pallidus*. These morphological differences support the hypothesis that *crystallinus*, *obscuripes*, and *pallidus* are unable to tolerate hydrostatic pressures greater than 0.5 atmosphere above normal, whereas *flavicans* is able to do so.

It is curious that three of the widely distributed species in Europe are restricted to shallow water, while none of the widely distributed species in North America is. The three stenobathic species are in two subgenera, and these subgenera are also found in North America. Apparently the gene mutations or recombinations that produce stenobathy arose on three independent occasions, and it was pure chance that all three took place in Europe.

TABLE 5. Lake categories in which the European species of *Chaoborus* have been found. Lake categories as in Table 1. Numerals refer to the number of lakes listed in Tables 2 and 3 in each category. Superscript numerals refer to supplementary information in the footnotes.

Species	Lake category			
	II	III	P	M
<i>crystallinus</i>	1 ¹		6	
<i>flavicans</i>	9 ²	2 ³	6 ⁴	1
<i>nyblaei</i>			1	
<i>obscuripes</i>			4 ⁵	
<i>pallidus</i>			6	

¹ See discussion in text of erroneous reports of this species.

² This is the only species known from Skären and Allgunnen, Sweden (Brundin, 1949), and Schöhsee in Germany (my data).

³ Found in small numbers in Innaren and Stråken, Sweden (Brundin, 1949).

⁴ Peus (1934) found this species only in inundation pools by rivers.

⁵ Prokešová (1959) found this species only in ponds.

⁶ Peus (1934) found this species only in pools, ditches, and ponds (rich in humus); Prokešová (1959) found it only in small pools; Edwards (1920) found it abundant in ponds (always in thick vegetation).

DISCUSSION

Coexistence of closely-related species can be explained in one of three ways: (1) their ecological niches differ, so that they do not compete, (2) the environment in which they live is not sufficiently stable to permit one species to exclude another by competition, or (3) there is no shortage of resources used by the coexisting species and hence no competition for these resources.

As mentioned earlier it is assumed that coexisting species of *Chaoborus* have niches sufficiently similar that they are potentially in competition with one another, based on the study of *flavicans* and *punctipennis* by Stahl (1966).

Coexistence of *Chaoborus* species in temporary ponds might be explained by the second alternative, but this seems an unlikely explanation for co-occurrence of species in the profundal zone of second-class lakes where environmental fluctuations and diversity of the habitat are notably low. The larvae do migrate up into the epilimnion at night, and there are seasonal variations in oxygen, but the seasonal minimum of oxygen is likely to be about the same from year to year. This explanation cannot be ruled out, but it seems unlikely.

Hutchinson (cited by Miller, 1964) pointed out that the competitive exclusion principle need not apply if the resources shared by coexisting species are not in short supply. Therefore the third explanation of coexistence is the most attractive. Miller found in the laboratory that the larvae of two closely-related species of *Drosophila* coexisted without adverse effects on each other as long as the density was sufficiently low and the cultures were started with equal numbers of each species. There is difference of opinion on how often populations in nature are at a saturation density. For example, Mayr (1963, p. 88) says, "Many of the resources of the environment are limited," whereas Andrewartha and Birch (1954, p. 140) say, "It is the lot of the great majority of species to remain rare relative to their food and other resources."

There is evidence that in some instances *Chaoborus* larvae may reach a saturation density. In second-class lakes when overwintering larvae migrate toward the deepest part of the lake, the population may become very dense. Some examples are: 43,456/m² in Myers Lake (Stahl, 1966), 69,300/m² in Lago di Varese (Bonomi, 1962), 93,940/m² in Lake Beloie (Borutzky, 1939), 97,000/m² in Linsley Pond (Deevey, 1941). In the three arms of Lago di Varese the larvae are smallest in the arm with the densest population. Bonomi attributes the reduced size to intra-specific competition. In Lake Beloie 50% of the population died between November and April, apparently due to over-crowding. Nevertheless these high densities do not necessarily mean that the larvae are competing with each other, because they probably do not feed while in the bottom, but only when they migrate up into the water (Berg, 1937; Deonier, 1943). Furthermore, the low temperatures in winter might slow the rate of feeding significantly.

Of the various factors that limit *Chaoborus* populations, predation by fish is doubtless one of the most important. It probably is the reason that the larvae are abundant only in second-class lakes in which the hypolimnion becomes anaerobic or nearly so and in small ponds lacking fish. In the

profundal zone of Esrom Lake there is little predation on *Chironomus anthracinus* during summer stagnation, but considerably more during overturn (Jónasson, 1965). Presumably this would be true for *Chaoborus*, also, which lives in the same environment (although it migrates into the epilimnion at night).

Even in second-class lakes in which the hypolimnion becomes anaerobic and in which *Chaoborus* is the dominant component of the profundal benthos, there is a tenfold or more variation in the maximum density of *Chaoborus*, indicating that other factors also affect the population density. One of these is success of reproduction. In *Chironomus anthracinus* from Esrom Lake, for example, the weather at the time of emergence may have a very important influence on the initial size of the larval population (Jónasson, 1965). This might apply to *Chaoborus*, too, although its emergence period is not so restricted. There is no clear evidence that the abundance is significantly different in lakes having coexisting species than in lakes having only one species. The apparent absence of competitive exclusion in *Chaoborus* in itself implies that significant inter-specific competition does not occur between coexisting species.

There are 12 lakes listed in Table 1 in which *flavicans* and *punctipennis* occur together and from which more than 100 specimens were identified. In all but one lake *punctipennis* formed a higher proportion of the population than *flavicans*. The exception—Redmond's Pond near London, Ontario—was the only pond containing these two species. More ponds must be sampled before deciding whether this difference in ratio is causally related to the lake category. The low proportion of *flavicans* relative to *punctipennis* does not indicate that *flavicans* is in the process of being excluded by competition, because no lakes within its range that have been adequately sampled lack this species (Table 1).

Much more needs to be known about the factors controlling the distribution and abundance of *Chaoborus*. The evidence presented here suggests that interspecific competition is not significant in this respect.

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The Change from Endogenous to Exogenous Sources of Energy in Bluegill Sunfish Larvae¹

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ABSTRACT

The numerical strength of a year class of fish is probably established before metamorphosis from larvae to adult. Factors investigated that affect the survival of bluegill sunfish larvae, *Lepomis macrochirus*, are the characteristics of the ova, fertility, hatching, and the events marking the "critical period" when the larvae switch from endogenous to exogenous nutrition.

Mature unfertilized bluegill ova are pale yellow, average about 1 mm in diameter, and stick readily to a substrate after stripping. Atretic ova were observed upon stripping eggs from females held in the laboratory and from a few fish trapped in lakes. These ova exhibit erosion of the yolk, have low viability, and do not adhere well to a substrate. The fertility and hatchability of mature bluegill ova varied from 80 to 90% in the laboratory. Ova nearest the urogenital vent had a higher hatching percentage than more anterior ova.

No significant mortality of eggs or larvae was caused by visible light from fluorescent bulbs. At 23.5° C in constant light, the mean hatching time was about 45 hours after fertilization. However, constant light lengthened the hatching interval by three hours.

In order to define the critical period, observations were made daily on the morphology and behavior of larvae from artificially fertilized eggs, and the larvae were sampled daily to follow their protein and energy metabolism. The onset of feeding and a decline in the dry weight, protein content, and energy content of the bluegill embryo marked the beginning of the critical period, 6.4 days after fertilization (4.4 days after hatching).

Between 8.5 and 9 days, starvation was manifested by a diminution of feeding success and food-seeking movements, a cessation of gain in total length, and the time of 50% mortality. These characteristics were used to define the end of the critical period.

The above criteria and others, such as the disappearance of the yolk sac and the diminution of total protein and total energy, are discussed in an effort to establish criteria that could be used to define the critical period for larval fishes in general. No one characteristic, single or in combination with others, was found to be valid for the demarcation of the critical period in all species.

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TABLE OF CONTENTS

	Page
Introduction	116
Methods	117
Results	122
Description of the larvae	122
Similarity of eggs within the ovary	123
Factors influencing fertility and hatching	125
Life history of starved larvae	129
Larval feeding	134
The metabolism of the larvae	137
Discussion	141
Evaluation of attributes of starved larvae in relation to the critical period	142
The metabolism of starved larvae in relation to the critical period	143
Acknowledgments	145
References	145

INTRODUCTION

The fluctuation of year classes in fishes is of great economic importance to the commercial and sport fisheries of the world, but very little is known about the causes of this fluctuation. The abundance of a year class of fishes is established shortly after spawning. Once established, the numerical strength of a year class continues in approximately the same relation to that of other year classes for the duration of its existence. Relatively small year classes of marine fishes may result from unusually high mortality of ova or larval fish (Hjort, 1914).

After fertilization of the egg, the causes of mortality can be categorized as follows: (1) predation, (2) disease and parasitism, (3) environmental stresses such as low oxygen tension, (4) embryonic anomalies, and (5) lack of food at the time the fish loses its yolk sac and turns to an exogenous food source. The last cause has commanded much attention by fishery biologists, partly because early workers such as Hjort (1926) argued rather convincingly that high mortality of larval marine fishes would occur if the larvae hatched some distance from a rich plankton source. Fabre-Domergue and Biétreix (1897) noted high mortalities of marine fish larvae in the laboratory during yolk sac absorption, presumably when the fish first take food. The consensus slowly emerged that the period when a fish switches from endogenous energy to exogenous energy sources, the "critical period," is extremely important in the formation of an abundant year class. Predation, disease, parasitism, environmental stress, and embryonic anomalies might be as important as the "critical period" during the early life of the fish, but their significance is beyond the scope of this paper. An attempt will be made here to define the length of the critical period wherein bluegill sunfish larvae (*Lepomis macrochirus*) must succeed in capturing exogenous food to insure survival. The criteria used in this respect are compared with other fish larvae.

Blaxter and Hempel (1963) defined the critical period for several races of the Atlantic herring, *Clupea harengus*, using the disappearance of the yolk sac, time of 50% mortality, and cessation of feeding movements as criteria. Many of their criteria are ecological in nature and have been used in this paper. However, it was felt that proper definition of the

critical period required also the use of certain aspects of the metabolism of the eggs and larvae, notably the use of protein and energy during development.

Various approaches have been used to study catabolism of protein and heat production in larval fishes. Smith (1947) measured loss of protein by the total nitrogen excreted by rainbow trout larvae (*Salmo gairdnerii*). Since nitrogen is 16% of protein, he could easily estimate the amount of protein catabolized. Energy loss between two periods of development was inferred from the difference in energy between two samples of larvae burned in a calorimeter. The loss can also be estimated by measuring oxygen consumption at various times during development (Lasker, 1962). When the oxygen consumed is known for a period of time, the energy lost during that period can be obtained, since the oxygen consumed is roughly proportional to the energy released. In this study, the Kjeldahl and calorimeter techniques were chosen to study protein metabolism and energy expenditure, respectively. Since the ova and larvae of the bluegill are very small, 200 or 500 ova or larvae were pooled for analysis.

Changes in the metabolism, morphology, and behavior of the larvae (embryos plus yolk sacs) are used to define the critical period. The inception of the critical period is that time when larvae begin to feed. Beyond this point the larvae must capture exogenous food or die of starvation. The effects of starvation are manifested at the end of the critical period. A diminution of feeding success, a decrease of food-seeking movements, and the time of 50% mortality establish this point.

While the definition of the critical period was the main purpose of this study, data were also gathered on the fertility and hatchability of fertilized ova, the effect of light on hatching at constant temperature, ova resorption, and the similarity of ova within the ovary.

The bluegill has tremendous reproductive success in Indiana lakes. Mature ova are distributed uniformly within the ovary, so that a tremendous number are capable of being spawned from one female at one time. The fertility and hatchability of bluegill ova are very high (80 to 90%) in the laboratory, and this is probably also true in nature. Reproduction is successful in spite of a relatively short critical period, which at 23.5°C begins 6.4 days after fertilization (4.4 days after hatching). Starvation clearly manifests itself between 8.5 and 9 days by the decreased feeding success, decreased food-seeking movements, and a marked increase in the rate of mortality. A critical period of about two days appears to be easily surmounted by extensive, indiscriminate feeding on particulate matter and small plankton. Bluegill larvae in lakes and ponds rich in these foods would have little trouble in surviving well past the end of the critical period.

METHODS

Source of material. Most of the ripe adult bluegills used as the source of eggs and sperm were seined or trapped from Sylvan Lake in Noble County, Indiana, from early June to early August 1964. Some adults were taken from Shriner Lake in Whitley County, Indiana, in July 1964.

The fish were transported to the Indiana University Biological Station on Crooked Lake, Noble County, Indiana, where the females were held from 2 to 24 hours before they were stripped of their eggs. In some cases the males were held longer than 24 hours. If females were held 2 or more days, their eggs were no longer fertilizable. Apparently yolk resorption occurred, as the eggs appeared pale and watery.

Fish larvae were taken from nests occupied by male bluegills in Dream Lake, Kosciusko County, Indiana. A large syringe, fitted with a long piece of hollow glass tubing, was used to remove the larvae from nests on 18, 19, 21, 22, and 23 June 1963. None were found on the nests on the 24th. The larvae were preserved in 10% formalin.

Temperature. Larvae were reared in a constant temperature room at the Biological Station. The temperature used throughout this research, 23.5°C, was lower than the minimum temperature at which bluegills spawn, 27°C, according to W. F. Childers (personal communication), but water temperatures in nature often drop after spawning has begun. Hunter (1963) found that water temperatures often fall below 23.5°C in small ponds after the spawning of a closely related species, *Lepomis cyanellus*. Bluegill larvae at 23.5°C gained 1.86 mm in length from the time of hatching until they became free-swimming and only 0.51 mm thereafter. Larvae taken from nests in Dream Lake occupied by male bluegills hatched between 17 and 18 June 1963 and left the beds between 23 and 24 June. The total increase in length of these larvae was 1.44 mm during 6 days in nature or 0.42 mm less than during a comparable 4.4-day period for larvae at constant temperature.

Ovary dissection. Fertility and hatching time were used as criteria to determine the maturity of eggs in different parts of the ovary. The ovary was first exposed, and four small slits were made in the serosa of each lobe. Small samples of about 200 eggs were withdrawn with separate pipettes from the posterior-medial, middle-medial, and anterior-medial portions of each lobe. Some of the eggs were placed in water in a petri dish and fertilized immediately with minced testes. Others were placed in an aluminum pan for dry weight measurement. Eggs were also taken from the peripheral portion of each lobe of the ovary from a second female. Four series of determinations of percentage fertility and percentage hatching were thus obtained from the bilobed ovaries of two females from the posterior-medial, the middle-medial, and the anterior-medial positions; two were obtained from the peripheral position.

Eggs dried to constant weight were used to determine the caloric content and the nitrogen content of eggs from various positions in the ovary. Eggs taken from both lobes of the ovary of one female were used for caloric analysis. Eggs taken from both lobes of the ovary of the other female were used for nitrogen analysis. In this case, eggs were taken from all the positions noted above except from the peripheral positions. Average caloric and nitrogen contents were then computed from eggs taken separately from each position in each lobe of the ovaries of the two females.

Measurements. Measurements were taken of the total larval length, the length and height of the yolk sac, the diameter of the oil globule, the width of the mouth, the length of the upper and lower jaw, and the dia-

meter of spherical unfertilized eggs with a binocular microscope fitted with an eyepiece micrometer. Such data were collected daily for as many days as the larvae lived and the structures continued to be present. Observations on at least 20 larvae were made each time. Measurements of lengths of larvae taken from nests in June 1963 from Dream Lake were made in August 1964. A correction factor (3.8%) for shrinkage of preserved fish was calculated by measuring the fish alive and preserved in formalin.

Fertilization procedure. The same fertilization procedure was used in all cases. One male was used to fertilize all the eggs of any one female. Both sexes were taken from the same lake whenever possible and paired according to length.

Eight 8-inch finger bowls, eight 6-inch petri dishes, or a combination of both were numbered and placed on a table in a linear fashion. Then the bottoms of the dishes were covered with a thin layer of unfiltered lake water. Three aluminum pans labeled A, B, and C were placed on the table next to the dishes. These pans received only eggs for the determination of dry weight and nitrogen content.

Both the male and female bluegill were weighed and measured separately. The male was then killed and its testis removed and placed in a watch glass. By gently squeezing the abdominal wall of the female, from 10 to 500 eggs were stripped into containers in the following sequence: dish 1, dish 2, pan A, dish 3, dish 4, pan B, dish 5, dish 6, dish 7, pan C, and dish 8. The testis was minced in a watch glass, a small amount of water was added, and the suspension was pipetted onto the eggs in the dishes as fast as they were stripped from the female. The water in the dish turned milky white. The milky water was discarded after about one-half hour, and the eggs were rinsed in unfiltered lake water. The eggs had adhered to the dishes by this time. Indeed, if they had not adhered, the husbandry was immediately discontinued, as experience proved that development would not proceed normally.

Subsequent care of eggs and larvae. Dishes were kept in a dark or semi-dark room at a constant temperature of $23.5 \pm 0.5^{\circ}\text{C}$, which allowed egg development to proceed at approximately the same rate as in nature. Eggs and larvae were shielded to minimize any deleterious effects of light, and the dishes were covered with glass to prevent contamination by dust.

Lots were checked several times during the day, and dead eggs and larvae were removed. The water in each dish was changed at least once a day by lake water filtered through glass wool. A high surface-area-to-volume ratio in each dish was accomplished by just barely covering the eggs or larvae with water. Artificial aeration was not employed.

Fertility of the eggs. Data were secured periodically on fertility of eggs. A fertilized egg is defined here as one in which there is a space between the egg membrane and the embryo regardless whether the embryo is alive or dead. The total number of eggs was divided into three categories: (1) mature and fertile eggs, (2) mature but infertile eggs, (3) immature eggs (smaller ova). Results are expressed as the percentage of fertile eggs calculated both from the total number of eggs and from mature eggs only.

Hatching time, percentage hatching, and effect of light. The length of time to hatch and the effect of light upon hatching time were determined. Eggs were stripped into eight large petri dishes and fertilized in the manner described above. The dishes were rinsed of the sperm suspension after 0.5 hour, filled with new water, and the numbers of mature, fertile, and live eggs were counted. Each dish was covered by a petri dish cover glass; four were wrapped in aluminum foil and four were unwrapped. Both sets were placed in a constant temperature room at 23.5°C at the same time. All dishes were 0.5 meter from a bank of four cool white, Powertrue 72, Sylvania fluorescent lights.

Each dish was examined hourly from 30 to 60 hours after fertilization, and the total numbers of hatched fish were removed and counted. A hatched fish is defined here as one that has broken free from its egg membrane.

The same dishes used to secure data on percentage fertility were often used to secure data on percentage hatching. Results are expressed either as the percentage of all eggs that hatched or the percentage of mature, fertile eggs that hatched. Dishes used in the experiment above to determine the effect of light on hatching time were also used to determine the effect of light on percentage hatching.

Starvation experiments. Immediately after hatching, 15 lots of 20 larvae each were removed from the fingerbowl in which they had hatched, placed into 15 different petri dishes, and kept from food. Ten dishes were provided with unfiltered lake water, which was changed daily. This was routine procedure in the rearing of all the rest of the larvae used for the metabolic and behavioral studies. Five of these ten dishes were covered with glass and wrapped in aluminum foil. Five were simply covered with glass. The remaining five dishes, which were provided with lake water filtered through glass wool, were also covered with glass and aluminum foil. Water was changed daily.

All 15 dishes were kept at 23.5°C in the constant temperature room 0.5 meter from the same bank of four fluorescent lights mentioned above. Each dish was examined daily, the water was changed, and dead larvae were removed and enumerated. In this way, the point at which 50% of the larvae had died of starvation was ascertained.

Feeding experiments. Six days after fertilization, about 300 larvae previously kept from food were placed in a five-gallon aquarium and kept under constant illumination. The larvae were fed a commercial liquid fish food ("Liquifry"), fresh plankton, and a wheat-hay infusion. No attempt was made to keep the number of larvae or the concentration of food constant in any experiment.

The next day the alimentary canal of the fish was examined for food with a binocular microscope. On the same day, a second batch of larvae kept from food until seven days after fertilization was also placed in another aquarium and fed the same food under identical conditions of temperature and light. This procedure was repeated for larvae kept from food for 8, 9, 10, and 11 days, respectively. Brine shrimp nauplii, *Artemia* sp., were offered on the last three days.

The number of larvae examined in each experiment varied from 28 to 290. A feeding larva was designated as one that had one or more food

items in its alimentary canal at the time of examination. An attempt was made to identify the item whenever possible. The results are expressed as the percentage of the total number of larvae that had fed.

Behavior during starvation. A group of starved larvae was observed daily in an aquarium kept at 23.5°C and under continuous illumination. Behavior before and after the free-swimming stage was observed. The position of the larvae relative to the bottom of the aquarium was observed, and the number of horizontal and vertical movements per minute was counted. Five or more observations were made each day, but it was not known if observations were made on the same or different individuals.

The procedure for the above was as follows. A larval fish was sighted, its position recorded, and an interval timer was set for two minutes. During that interval, the number of pauses on the bottom and the number of pauses above the bottom were recorded. Also recorded was the number of times the larva moved horizontally along the bottom (1 cm or less above the bottom), and number of horizontal and vertical movements. A horizontal movement was one in which the horizontal component was greatest, while a vertical movement was one in which the vertical component was greatest. The results are averaged and expressed in units per minute.

When the larvae became free-swimming, the following factors were also measured: (1) swimming speed, (2) length of pause or drift between swimming movements, and (3) the duration of each swimming motion. In the case of swimming speed, a ruler was taped to the aquarium, and the position of a resting fish was noted. If the fish moved parallel to the ruler, its duration of movement was timed with a stopwatch from start to stop, and the distance moved was recorded. Speed of swimming is the average distance moved divided by the average time spent in movement. Length of pause, which was timed with a stopwatch, is defined as the time between the end of one swimming movement and the beginning of another. The duration of each swimming motion, regardless of direction or distance, was also timed between the start and the end of a swimming motion. Five or more observations were made daily for each of the above categories, but it is not known if the same or different individuals were observed.

Sampling procedure for metabolism studies. In order to determine the daily change in dry weight, in the caloric content, and in the total nitrogen content of both the living material and the yolk of the embryo or larva from the time of fertilization to the time at which the last larva died, samples of unfertilized eggs as a basis for comparison were taken in aluminum pans as was described under the fertilization procedure. The eggs were fertilized, and on each succeeding day, 2 to 6 samples of 200 to 500 unhatched eggs or larvae were sacrificed, counted, and placed into aluminum pans, each previously weighed to the nearest 0.01 mg. This material was dried and weighed in the manner described below. Two samples of 200 to 500 unhatched eggs or larvae were sacrificed, counted, and placed on asbestos. The material in aluminum pans was used to obtain data on dried weight and caloric content, while the material on asbestos was used for the analysis of total nitrogen. Dried eggs and larvae in aluminum pans or on asbestos were preserved 1 to 6 weeks in a

deepfreeze before they were used for analysis. It was necessary to combine eggs from more than one female to obtain the number in each sample required for analysis.

The material in the pans was dried to constant weight for 48 hours in an oven at 38°C. Eggs and larvae were analyzed for their energy contents with a Parr adiabatic, oxygen bomb calorimeter. Total nitrogen content of eggs or larvae was determined by a steam distillation, rotary, electrically heated micro-Kjeldahl apparatus (American Instrument Company). A known weight of unfertilized eggs or a known number of unhatched eggs or larvae was digested according to the method suggested by Brüel *et al.* (1947). Known weights of acetanalide treated like the samples served as controls for each day's distillation. The nitrogen content of unfertilized eggs is expressed both in terms of egg weight and egg number, but the nitrogen content of developing eggs or larvae is expressed only in terms of amount of nitrogen per individual.

RESULTS

Description of the larvae

The description of the embryo prior to hatching has been made by Morgan (1951) and will receive no attention here. However, certain features of the starved larvae will be described. Detailed observations with a binocular microscope supplemented by those made with a compound microscope were made daily for 9 days after hatching. Age in days and in hours after fertilization identifies the fish in the description below. One fish was observed on each day. However, the data on length range were obtained by measuring many larvae from many different parents.

Two days (56 hours after fertilization). The total length ranged from 3.26 to 3.72 mm. The alimentary canal was apparent from the yolk sac to the anus. Optic vesicles and lens of the eye were present. The auricle of the heart was in front of the yolk sac and beating. The blood was red. Tiny pectoral fins were apparent above the yolk sac. A continuous finfold started just above the posterior end of the yolk and extended around to the anal region. The yolk was lemon shaped. One oil globule was located within the yolk sac.

Three days (73.5 hours after fertilization). The total length ranged from 3.48 to 4.40 mm. The yolk sac was smaller, but still lemon shaped. The yolk appeared to be made up of a large number of blocky units. The anus was clearly open.

Four days (97.0 hours after fertilization). The total length ranged from 4.27 to 5.54 mm. The alimentary canal was clearly open from above the yolk sac to the anus and appeared yellow-green. The jaws were present, but the mouth was not open. The eye was pigmented. Blood could be seen flowing along the gill arches. Faint rays appeared in the pectoral and caudal fins. The caudal fin was homocercal, but the continuous finfold was still present.

Five days (129 hours after fertilization). The total length ranged from 4.64 to 4.82 mm. The mouth was open, and the jaw moved weakly.

The swim bladder was clearly evident. The pectoral fins beat very rapidly.

Six days (148 hours after fertilization). The total length ranged from 4.58 to 5.21 mm. The alimentary canal was lined with large yellow endodermal cells and was clearly open from the pharynx to the mouth. Peristalsis was evident. The pectoral fins were in the shape of large fans, which moved rapidly. The continuous finfold was reduced except in the tail region. Mesenchyme cells were present in the dorsal and anal fins. A pigment cell was located on the side of the body just dorsal to the anus.

Seven days (169.5 hours after fertilization). The total length ranged from 4.93 to 5.74 mm. Larvae were now free-swimming. The yolk sac was smaller. The oil globule was now in the anterior part of the yolk.

Eight days (200 hours after fertilization). The total length ranged from 4.99 to 5.81 mm. Yolk was almost gone, and the larvae were now clearly "pinheads" with a large head and narrow trunk. Dorsally the finfold had almost disappeared. Pigment cells were present in pairs lateral and dorsal to the anus, along the abdomen, and posterior to the liver.

Nine days (217 hours after fertilization). The total length ranged from 4.92 to 5.84 mm. The yolk was gone except for a tiny oil globule. The pelvic fin was present. The swim bladder was black and appeared to be composed of many vacuoles. The skull appeared to be chondrifying.

Ten days (238 hours after fertilization). The total length ranged from 5.41 to 5.80 mm. The tiny oil globule was still present. The swim bladder was dark, the vacuoles appearing to have coalesced. The caudal fin had more fin rays, and the continuous finfold was reduced. The lens of the eye appeared orange.

Eleven days (262 hours after fertilization). The total length ranged from 5.40 to 5.63 mm. The oil globule was gone. The effects of starvation were evident. The larvae were very thin, with a marked constriction in the abdomen. On each side of the body about 11 pigment cells were present between the ventral somites from the anus to the tail; 2 to 3 were lateral and dorsal to the anus, and 3 to 4 were on the ventral part of the abdomen between the heart and the anus. The swim bladder was conspicuously pigmented.

Similarity of eggs within the ovary

The size of year classes of the bluegill may be influenced by the fertility and hatchability of eggs. In order to discover whether all eggs are at the same stage of development in a single female, representative samples were taken from different parts of the ovary. Preliminary studies indicated that mature eggs were not randomly distributed in an antero-posterior direction within the ovarian lobe of the bluegill. In one egg-stripping experiment, the first eggs to emerge were more fertile than those that emerged last, but in another experiment the reverse was true. Stripping was obviously of little use in the determination of the degree of maturity of eggs within an ovary, since the ovarian position of the eggs could not be determined. To overcome difficulties in the stripping technique, eggs were removed with a pipette from precise areas within the right and left ovarian lobes of two females after opening the body cavities.

These eggs were analyzed for their nitrogen content, caloric content, and dry weight. Their maturity also was measured by their fertility and hatchability.

The ovary of a ripe bluegill sunfish is a bilobed elongate organ lying against the ventral wall of the swim bladder in the postero-dorsal part of the body cavity. The axis of each lobe makes a slight positive angle with the body axis. Each lobe is circular in cross section and lobate in either frontal or median section. Many ovigerous lamellae project into the center of the ovary. The posterior ends of each lobe are fused into the oviduct, which continues about 1 cm posteriorly and ventrally to the urogenital sinus.

The right and left lobes of the bluegill ovary were assumed to be at the same stage of maturity. Yuen (1955) showed this to be true for the ovarian lobes of the bigeye tuna, *Thunnus obesus*, and MacGregor (1957) demonstrated it for the ovarian lobes of the Pacific sardine, *Sardinops caerulea*.

Nitrogen and energy content of eggs. The nitrogen content of medial eggs was about 10% of the dry weight or about 2 μg N/ovum. If the one low value for eggs in the middle-medial position is ignored, then on the average there is very little difference in the nitrogen content of the eggs regardless of their antero-posterioral position (Table 1).

TABLE 1. Dry weight, energy content, and nitrogen content of ova from different position within the ovary (R = right lobe, L = left lobe, A = female A, B = female B, * = not included in averages).

Position	Code	Dry wt. (μg)	Energy content (cal/ovum)	Nitrogen content (μg N/ovum)
Posterior-medial	RA	184	—	1.69
	LA	176	—	1.96
	RB	139	0.873	—
	LB	135	1.088	—
	Av.	159	0.981	1.85
Middle-medial	RA	172	—	0.64*
	LA	184	—	1.94
	RB	140	0.747	—
	LB	135	0.782	—
	Av.	158	0.765	1.94
Anterior-medial	RA	195	—	2.04
	LA	180	—	1.93
	RB	138	0.803	—
	LB	138	1.249	—
	Av.	163	1.026	1.99
Average of all medial positions		159.7	0.924	1.91
Periphery	RB	144	0.820	—
	LB	157	0.790	—
	Av.	150.5	0.805	—

While medial ova were quite alike in nitrogen content, the lamellar folding of the bluegill ovary suggested that peripheral eggs would be different from medial eggs, since the more mature ova would be on the outer margin of the lamellae toward the medial portion of the ovary. No data were available to make such a comparison for the nitrogen content of peripheral and medial ova, but data on the caloric content and dry weight were obtained. Ova from the periphery had an average caloric content of 0.805 cal/egg compared with an average of 0.924 for eggs from the medial positions. A "t" test of unequal numbers of observations (Snedecor, 1956, p. 90) between the energy content per egg of ova from the periphery and ova from the medial positions showed there was no significant difference ($t = 0.804$, 6 *d.f.*, $p = 0.2$).

Peripheral ova were about 9 μg less than all medial ova on the basis of the dry weight per egg. The same statistical procedure as above was used to test for the difference between these groups. Ova on the periphery weighed 150.5 μg ; those medial weighed 159.7 μg . The difference of 9.2 μg was not statistically significant ($t = 0.532$, 11 *d.f.*, $p = 0.5$).

Fertility and hatchability. A comparison of fertility and hatchability of ova from the medial and peripheral positions demonstrates sharply that the peripheral ova are much less mature than those located in a medial position. Roughly twice the percentage of immature ova were taken from the periphery as from medial positions. Thus, when percentage fertility and percentage hatching were computed from the total number of eggs taken from any position, the results were much lower than if these data were computed from the number of mature eggs alone. The medial eggs from a variety of positions in the ovary were alike with regard to fertility and hatchability (Table 2), but there was a statistical difference between peripheral eggs and medial eggs. Chi-square values were computed in an R X 2 table (Snedecor, 1956, p. 227) as follows:

	Total ova	No. fertile	Ratio
Medial	2343	1730	0.738
Peripheral	1137	510	0.449
Chi-square = 276 with 1 <i>d.f.</i> $P = <0.005$			

Moreover, chi-square was 243.5 ($P = >0.005$) for the comparison of the hatchability of peripheral ova, (12.8%) and medial ova (34.7%) from all positions using a similar test.

There was a suggestion that ova nearest the urogenital sinus, *i.e.* posterior-medial eggs, had slightly better hatchability, 41.0%, than those in other medial positions, 31.5%. Chi-square was 22.9 (1 *d.f.*) with a 0.005 probability of a larger value. The ova nearest the sinus were thus more mature as measured by hatchability than ova in the middle-medial and anterior-medial positions as a group.

Factors influencing fertility and hatching

The viability of the male gametes was not considered to be an important factor influencing the percentage of eggs fertilized because of the generous amounts of minced testes used in each dish. But this assumption

should be subjected to considerable scrutiny. W. F. Childers (personal communication) of the Illinois Natural History Survey, has shown that the viability of the male bluegill gametes decreased markedly after suspension of one minute in tap water. While fertilization of eggs using the crude techniques reported here was accomplished in a very short period of time, and fresh testes were minced several times during the operation, the viability of male gametes may have been reduced in some instances.

Characteristics of the eggs. The average diameter of 100 spherical unfertilized ova was 1.04 mm. Twenty eggs from each of five fish were measured. A conspicuous oil globule is invariably located on top of the egg at the polar end opposite the substrate.

Upon fertilization the cytoplasm shrinks, and the space between the egg membrane and the yolk enlarges. The membrane is at first soft and pliable, but it rapidly hardens. Concomitant with this event is the movement of cytoplasm to the side of the oil globule on the polar end of the egg opposite the substrate to form the blastodisc. The color of blastodisc and the oil globule are faint yellow, while the yolk is bright yellow-orange. After about 0.5 hour the egg cleaves for the first time.

TABLE 2. Percentage fertility and hatching of ova from different positions within the ovary (R = right lobe, L = left lobe, A = female A, B = female B).

Position	Code	% fertility		% hatching	
		Total ova	Mature ova	Total ova	Mature ova
Posterior-medial	RA	66.9	85.9	25.4	32.6
	LA	89.9	94.2	75.2	78.8
	RB	74.9	95.7	37.0	49.4
	LB	64.2	97.7	36.3	56.5
	Av.	72.7	94.7	41.0	53.4
Middle-medial	RA	54.8	87.1	17.2	27.3
	LA	83.9	98.6	13.1	15.4
	RB	85.5	94.5	48.0	56.3
	LB	75.0	98.4	30.2	40.9
	Av.	74.5	94.6	28.9	36.7
Anterior-medial	RA	84.7	93.5	23.9	26.5
	LA	89.7	96.0	44.8	48.0
	RB	52.5	78.7	13.1	25.0
	LB	80.9	95.2	54.4	67.2
	Av.	74.7	90.9	34.1	34.4
Average of all medial positions		73.8	93.4	34.7	41.5
Periphery	RA	50.4	93.4	8.3	15.4
	LA	33.4	54.3	3.5	5.7
	RB	49.8	99.0	19.8	54.9
	LB	50.1	99.3	18.3	35.9
	Av.	44.9	89.4	12.8	23.3

Degree of female maturity. Ripe female bluegills held for more than two days in large holding tanks in the laboratory apparently resorbed their eggs. This condition may be due to trauma, or it may be a natural phenomenon. Some females caught at the end of a natural period exhibited resorption of eggs. Eggs taken from such females have less yolk than normal eggs; large oil droplets were more conspicuous in the resorbing egg than in the normal mature egg. However, the oil globule *per se* did not seem to change in shape or size. Gentle pressure on the sides of the abdomen of the females whose eggs were undergoing resorption resulted in a copious watery flow of eggs.

The eggs undergoing resorption did not stick to any substrate and were non-fertilizable. But in other cases, eggs with a normal appearance from an apparently ripe female also did not adhere to a substrate and were likewise non-fertilizable. There must exist, therefore, some optimal physiological maturation stage for bluegill eggs. It seems likely that in nature only eggs capable of immediate fertilization are laid.

Degree of ova maturity. The criterion used here for a fertilized egg—the appearance of a perivitelline space—is also a characteristic of an egg that has been activated parthenogenetically (Rothschild, 1956). Some eggs in the present study reported to be fertile may have been activated parthenogenetically, which may help account for the fact that the percentage of ova hatching was always considerably less than the percentage “fertilized” (Table 2). However, these differences more likely resulted from high mortality in those dishes contaminated by dead ova.

The number of immature eggs (small ova) obtained by stripping eggs from a female was probably greater than if the fish had spawned naturally. Therefore, when the values for percentage fertility of all eggs (immature and mature) are considered, the values (Table 3) are lower than those probably occurring in nature. But the percentage of fertility of mature eggs alone is high, 85.7 to 96.4%. Hestor and Smitherman (1962) reported a fertility of 76% for bluegill ova, but their criterion of fertilization was the presence of a blastodisc, and thus their results are not strictly comparable to mine. However, if only mature eggs are spawned in nature and if these are laid at the peak of physiological maturity, then a high fertility potential can be expected.

Hatched larvae were those that had broken free of their egg membranes. In some instances these larvae were dying at the time of hatching. Their tails were shriveled, and they swam very little. Some may have hatched precociously, since agitation of the water during handling may

TABLE 3. Fertility of ova stripped into petri dishes and fertilized with minced testes.

Experiment	Number of petri dishes	Number of ova			% fertile	
		Total	Mature	Number fertile	of total ova	of mature ova
1	8	1670	1235	1059	63.1	85.7
2	8	1810	1225	1189	65.5	96.4
3	8	1802	1736	1546	85.8	89.1

have caused premature rupture of the egg membrane. In the instances where 88.5% and 90.5% of the total eggs hatched (Table 4, MC-1, MC-2), the dishes were free of immature, decayable eggs from the start, while this was not so in other experiments. Thus, under these ideal conditions, a very high percentage of eggs hatched. Fouling is perhaps insignificant in nature, since the male continually fans the nest. However, even in the absence of the male, water movement over the nest may be great enough to provide dissolved oxygen and to carry away waste products. There was no mass mortality of newly hatched larvae when males were prevented from entering a nesting area in Dream Lake.

Light. The data on mean hatching time in semi-darkness and the data on the percentage of ova hatching in semi-darkness should not be compared to the respective data for ova kept in continuous light or in continuous darkness because of the possibility of severe fouling of the water in the dishes in semi-darkness. The absence of such fouling in dishes in continuous light and in continuous darkness, however, permits comparisons concerning percentage hatching and the time of hatching between these two situations. Using a chi-square test in an R X 2 table (Snedecor, 1956, p. 227), the average percentage of ova hatching in constant light ($P_i = 0.905$) was not statistically different from the average percentage of ova hatching in constant darkness ($P_i = 0.885$): chi-square = 1.36, 1 d.f., $P = 0.250$.

Visible light can cause mortality of salmonoid eggs. Radiation from 40-watt electric lights caused 90% mortality of eggs of the brook trout, *Salvelinus fontinalis*, when they were in hatchery troughs (Perlmutter, 1961). Eisler (1958) showed that radiation from fluorescent lights penetrating through glass and water killed ova and alevins of the chinook salmon, *Oncorhynchus tshawytscha*. However, visible light does not seem to have the same lethal effect on eggs of non-salmonoids. Belyi (1961) reported no significant difference in egg mortality between a group of perch-pike eggs (taxonomic status not given) kept in asphalt covered

TABLE 4. Hatching of ova in petri dishes at 23.5° C under various conditions of illumination.

Code for experiment	Illumination	Number of petri dishes	Number of ova			% hatching	
			Total	Mature	Hatching	of total ova	of mature ova
HA	semi-dark	8	1670	1059	331	19.8	31.3
LA	semi-dark	8	1810	1185	567	31.3	46.3
MA	semi-dark	8	1802	1546	525	29.1	33.9
MC-1	constant light	4	283	283	256	90.5	90.5
MC-2	constant darkness	4	303	303	268	88.5	88.5

dishes and a group of perch-pike kept in "crystallizing" dishes exposed to the radiation from a 10-watt electric light. Presumably these latter dishes in the light were covered with glass. There is some discrepancy then between results on salmonoid material and those on bluegill and pike-perch. This discrepancy may be due to differences in technique or due to inherent differences between species. The salmonoid fishes bury eggs in gravel, and thus their eggs may be very sensitive to light. The bluegill and the pike-perch do not bury their eggs, and hence their eggs may be less sensitive. The yellow-orange carotenoid pigments of bluegill ova might decrease penetration of radiation.

Eggs in the same petri dishes as above (Table 4, MC-1, MC-2) were used to establish the time when 50% had hatched. The mean hatching time for four dishes kept in constant darkness was 45.75 hours after fertilization; for the four dishes continually exposed to the light, it was 41.87 hours. The differences between the means, 3.88 hours, was statistically significant ($t = 5.77$, 6 *d.f.*, $P = 0.005$). Continuous light accelerated hatching in this experiment.

The first larvae hatched in the dark 37 hours after fertilization, while the last hatched at 60 hours. In constant light, the first larvae hatched 35 hours after fertilization, while the last hatched at 55 hours. The hatching period was 3 hours shorter, and the median hatching time was about 4 hours earlier in constant light than in constant darkness interrupted hourly by a brief exposure to light. Presumably light caused the larvae to move actively within the egg membrane, and this movement resulted in precocious hatching. Haempel and Lechler (1931) found that light accelerated hatching of ova of brown trout (*Salmo trutta*) and that it also shortened the hatching period.

Life history of starved larvae

Measurements were made of total length, yolk volume during development, loss of dry weight, larval mortality, and the feeding movements while the larvae were kept from food. These observations were made in an effort to define the "critical period" when larvae switch from endogenous to exogenous nutrition.

Total length. The total length of larvae kept from food increases exponentially during the first nine days of development and then levels off (Fig. 1). The slight length decrease on days 10 and 11 may be due to the fact that the eggs from which these larvae hatched were smaller than the eggs that gave rise to larvae measured on the ninth day. Blaxter and Hempel (1963) showed that at hatching larger eggs produced longer larvae than smaller eggs in the Atlantic herring. Moreover, different larvae were measured each day in the present case. Although each day's average was computed from measurements on 40 fish, there may have been enough variability to account for the slight decrease on days 10 and 11.

Yolk sac volume. The equation $V = \pi/6 Lh^3$, where V = volume, L = length of yolk sac, and h = height of yolk sac, was used to compute the yolk sac volume (Blaxter and Hempel, 1963). The oil globule is included in these volumes, but since it persisted longer than the yolk sac, its volume was calculated using the formula for the volume of a sphere.

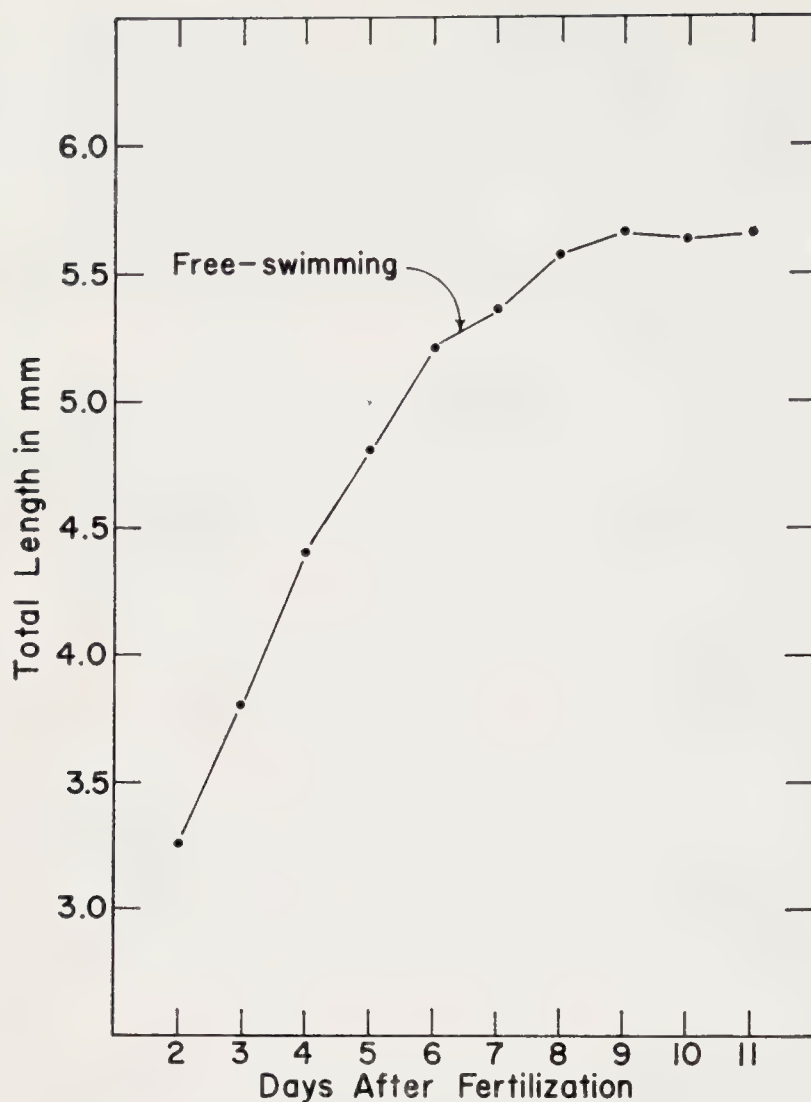


FIG. 1. Larval lengths between hatching and 11 days.

The volume of the unfertilized egg was also calculated as the volume of a sphere.

At hatching, 438 μl remained of the initial 603 μl of yolk (Fig. 2). About 28% of the original volume had been lost. The loss of yolk was highest between days 4 and 5, and thereafter it decreased. By the free-swimming stage (6.4 days), only 54 μl of the original 603 μl remained, or 9.2% of the original yolk volume. All of this yolk, except a tiny oil globule, 0.01% of the original volume, was gone by 9 days. These data clearly show that most of the yolk (89.5%) disappeared prior to the free-swimming stage and that very little was present thereafter.

Apparently the oil globule, which persisted to about 2 days after the yolk sac disappeared, provided some of the energy for swimming. There is reason to suspect that the oil globule was mainly triglyceric fat. These are the last reserves to be metabolized (Smith, 1957), which would probably account for the persistence of this feature.

Starvation experiments. The disappearance of the yolk sac between 8 and 9 days after fertilization corresponded to the time when 50% of

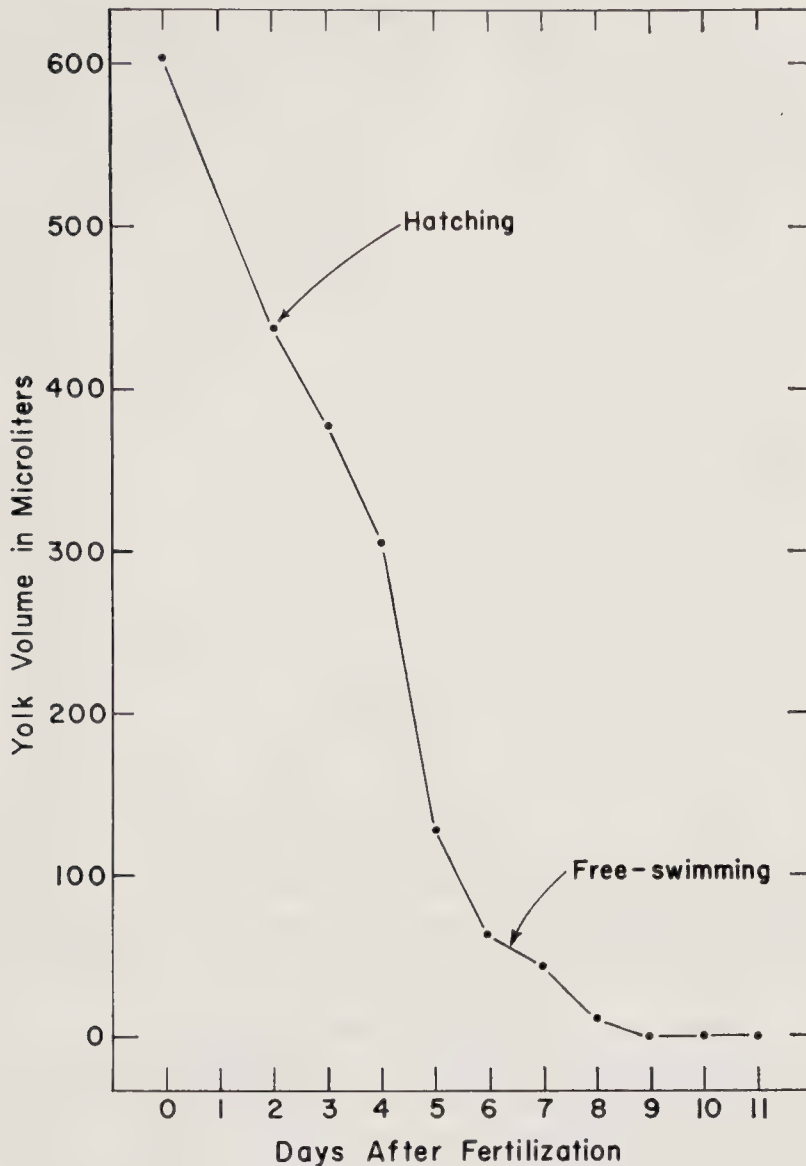


FIG. 2. Yolk sac and oil globule volume between fertilization and 11 days.

larvae kept from food had died. This was true both for larvae kept in constant light and for larvae kept in constant darkness (Fig. 3). Thus, 50%-survival time was roughly 6 days after hatching.

It is not known if the disappearance of the yolk sac or cessation of length gain at 9 days had any causal relationship to the time when 50% of the larvae died. The rate of mortality increased sharply after 8 days. More than half of a population of bluegill larvae kept from food in the laboratory or denied food by chance in nature would have been dead between 8 and 9 days. The rest of the population would be faced with the serious problem of finding food to escape death. On the basis of these starvation experiments, the end of the critical period has been fixed as the time when 50% of larvae kept from food had died of starvation (about 8.5 days after fertilization).

Swimming movements. Bluegill larvae at 23.5° C became free-swimming at about 153 hours after fertilization. Over a one-minute

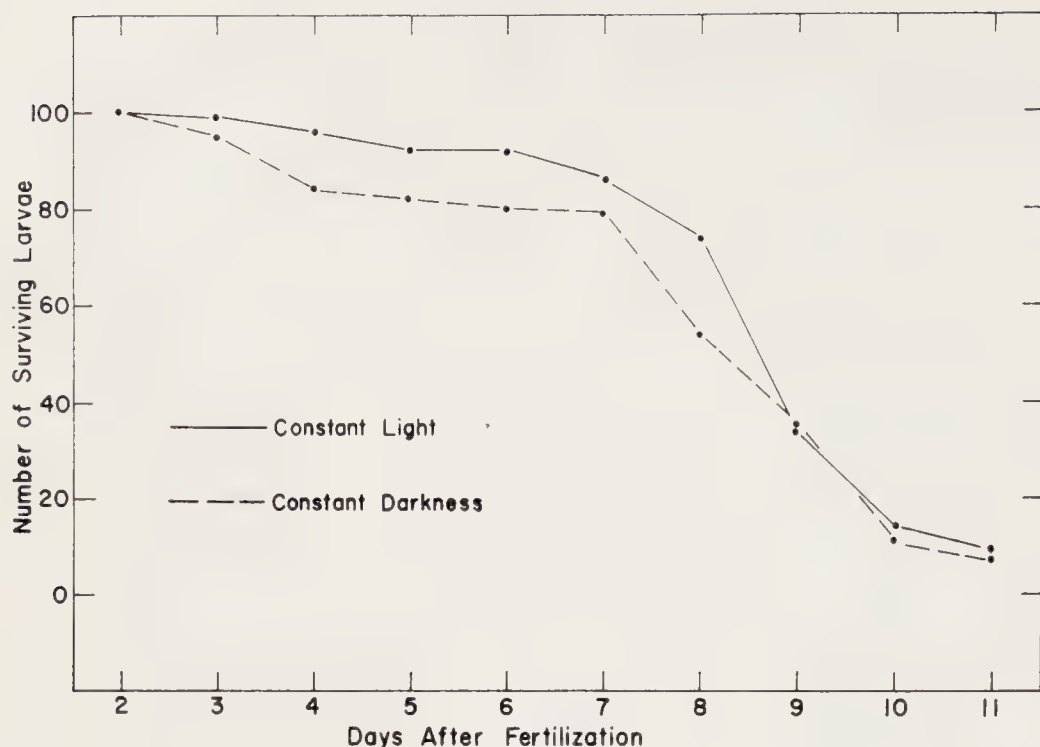


FIG. 3. Number of surviving larvae after hatching at 2 days.

interval larvae were free-swimming for 4.45 sec. at 144 hours, 5.00 sec. at 149 hours, and 50.5 sec. at 153 hours. Horizontal movements were 1 cm or less above the bottom of the aquarium before 153 hours (6.4 days), but thereafter, horizontal movements were all 1 cm or more above the bottom. Also, up to 153 hours larvae rested upon the bottom of the aquarium, but all of the rest stops were 1 cm or more above the bottom after 153 hours. Prior to the free-swimming stage, larvae made periodic vertical migrations to the surface of the water in the aquarium, which became more frequent as the free-swimming stage was approached.

The duration of swimming periods decreases while the duration of rest periods increases as the larvae get older (Fig. 4). The number of horizontal movements decreases from about 20 per minute to less than 2 per minute between 10 and 11 days. Thus, as the larvae starve, there is an overall decrease in the duration and number of food-seeking motions. This decrease became apparent between 8 and 9 days, which also marked the cessation of growth, the loss of the yolk sac, and the time when 50% of larvae kept from food had died. Thus, this decrease in swimming movements also signals the end of the critical period. Every movement used energy, but if no food was taken, every movement made the next less efficient. They had reached a point of diminishing return in the expenditure of energy.

Dry weight. There was considerable variability in dry weight of the eggs and embryos, the weights on days 1, 5, and 8 being lower than those on days 2, 6, and 9, respectively. The initial size of the egg on days 1, 5, and 8 may have been smaller, which would result in relatively smaller larvae on these days than on the other days. In an effort to overcome this variability, comparisons were made by using the percentage dry weight of the original egg that remained in the form of an egg or larva on the day of sampling.

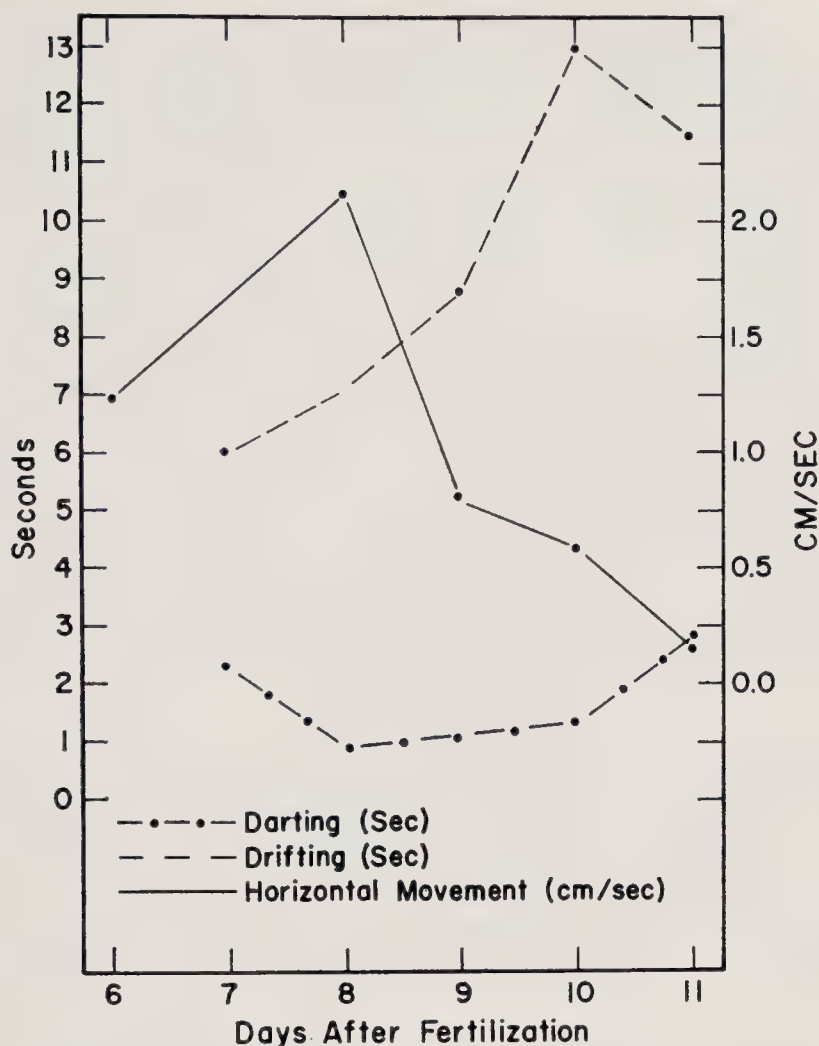


FIG. 4. Swimming behavior of starved bluegill larvae between 7 and 11 days after fertilization. Solid line indicates rate of swimming in cm/sec; dashed line is length of rest stops in sec; and dot-dashed line is length

The initial dry weight of the egg includes the egg membrane. The weight of the egg membrane was not determined for the bluegill, but it is probably a small percentage of the dry weight of the entire egg. Moreover, the average percentage dry weight of the egg membranes of other freshwater fish eggs is very small, being 1.23% for the carp and 1.37% for the pike (König and Grossfeld, 1913). The weight of the egg membrane was, therefore, felt to be insignificant in making the calculations that follow.

The initial average dry weight of the eggs was estimated from the average dry weight of eggs taken in the aluminum pan. For example, the weight of eggs in dishes 6, 7, and 8 was estimated from the weight of eggs in the pan that received eggs after dishes 6 and 7 but before dish 8 in the stripping procedure.

The average dry weight of developing eggs or larvae at any stage was determined by dividing their total weight by the number of eggs or larvae in the sample. The weight of each stage of egg or larva was converted to the percentage of the initial dry weight of the ova from which it had developed.

The percentages of the dry weight of the original ova remaining on the various days multiplied by 1000 mg yields the dry weight of larvae (embryos plus their yolk) remaining on those days for ova initially 1000 mg dry weight (Table 5, Column *B*). The weight of the yolk remaining on each day was estimated by calculating the percentage of the initial volume remaining on each day and by multiplying this percentage by 1000 mg (Table 5, Column *D*). The weight of the larvae less their yolk weight yielded the dry weight of the embryos (Table 5, Column *E*).

The embryos gained weight rapidly between fertilization and 5 days. The slight decrease on day 4 may be due to lack of precision in the yolk sac measurements, but the general picture remains the same. Growth is slower after 5 days, the embryos reaching their maximum weight at 7 days. After 7 days the embryos lose weight in a stepwise fashion, apparently losing tissue for catabolic purposes. The loss of yolk is so rapid that by the free-swimming stage (6.4 days) only 9.2% of the original yolk remains. Shortly thereafter, at 7 days, the embryos begin to resorb tissue to meet their catabolic demands. Between 7 and 11 days 214 mg is lost, or roughly one-third of the maximum weight at 7 days.

Larval feeding

The capacity to feed during the period when the larvae change from endogenous nutrition to exogenous sources of food is of great importance to survival. An attempt was made to show when the larvae first fed, when 50% or more fed, and the relationship between the size of the mouth and the size of possible food items.

The feeding of larvae offered food after various periods of starvation. Six days after fertilization about 200 larvae previously kept from

TABLE 5. Determination of the dry weight of embryos between fertilization and 11 days for 1 g dry weight of unfertilized ova (* = interpolated from Fig. 2).

	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>
Days after fertilization	Embryo plus yolk remaining (% initial ova)	Column <i>A</i> times 1000 mg	Yolk sac remaining (% initial ova)	Column <i>C</i> times 1000 mg	Weight of embryos remaining (Column <i>B</i> minus Column <i>D</i>)
0	100.0	1000	100.0	1000	0
1	94.3	943	86.2*	862	81
2	89.0	890	72.6	726	164
3	88.3	883	55.9	559	324
4	80.2	802	50.6*	506	296
5	76.8	768	21.1	211	557
6	68.7	687	10.5	105	582
6.4	68.0	680	9.2*	92	588
7	66.9	669	7.3	73	596
8	53.8	538	1.7	17	521
9	51.7	517	+	+	517
10	41.5	415	0.0	0	415
11	38.2	382	0.0	0	382

food were offered infusoria, fresh plankton, and "Liquifry." They were allowed to feed about 24 hours. At the beginning of the seventh day, they were examined for food items in their alimentary canals and preserved. A different group of larvae, previously kept from food, was offered food for about 24 hours between days 7 and 8. They were examined and preserved on day 8. The same procedure was repeated on days 9 and 10. A feeding larva was defined as one with one or more food items in its alimentary canal at the time of observation.

The percentage of larvae feeding was plotted against the midpoint of the period in which the fish were offered food. A curve connecting these points resembles a normal distribution (Fig. 5). Since the examination of large numbers of larvae took 4 to 6 hours, the feeding periods overlapped, *i.e.* 5.9 to 7.1 days, 6.8 to 8.1 days, and so on. The low percentage feeding between 141 and 171 hours after fertilization (5.9 to 7.1 days) was due to the fact that the larvae became free-swimming at about 153 hours (6.4 days). Examination of these larvae began at 162 hours, and not until about 168 hours were food items noted in their alimentary canals. During this time the larvae were in a shallow pan with little room to move about. The fact that in the next period, 6.8 to 8.1 days, 60.8% had ingested some food argues for the conclusion that they start

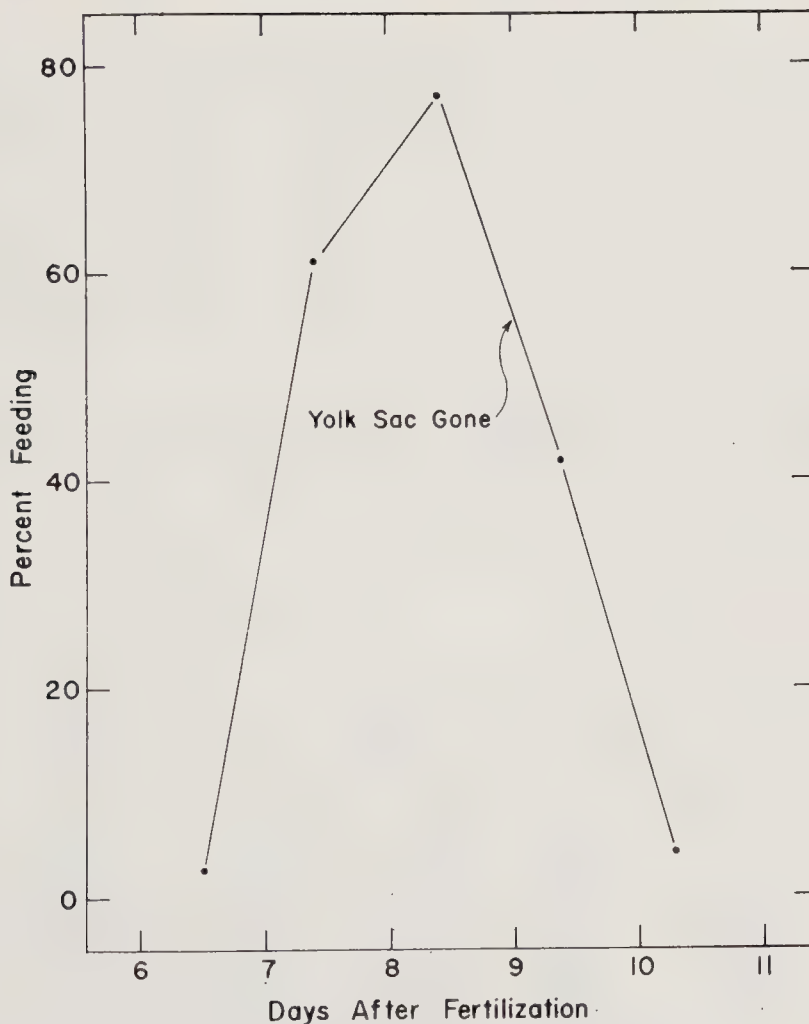


FIG. 5. Percentage of larvae feeding.

to feed shortly after they become free-swimming. Feeding marks the beginning of the critical period.

The feeding interval has been designated here as the time interval when 50% or more of the larvae had food in their alimentary canals. Extrapolation from the point where the curve crosses the 50% intercept establishes the feeding interval between 182 and 226 hours after fertilization (7.6 to 9.4 days). Although the feeding interval extended to 9.4 days, it must be remembered that about 66% of the population at hatching would have been dead by this time according to the starvation experiments (Fig. 3). Therefore, while a group of starved larvae can feed well at 9.4 days, they represent only a portion of the population.

It is of more concern to establish the time when fasted larvae may be so weak that they cannot possibly capture food even when it is abundant. This marks the end of the critical period. On the basis of the feeding experiment this must come between 8 and 9 days after fertilization for the following reasons. While it is clear that between 8 and 9 days feeding is most successful (76.4% fed at 8.8 days), very shortly after this time feeding success decreased sharply (27% fed at 9.8 days). Thus, feeding success sharply and continually decreases after 8.5 days from levels reached between 6.4 and 8.5 days. On this basis it is likely that survival-dependent feeding success was highest between 6.4 and 8.5 days for most of the population.

Feeding in relation to mouth size. The sizes of the upper and lower jaws of the larvae increased in size after day 5 when the mouth opened (Table 6). Width of the mouth, as measured between the articulating edges of the lower jaw, decreased after 8 days.

Bluegill larvae feed in aquaria by moving about with their mouths open. It was observed that larvae in aquaria hold their mouths open such that the angle between the upper and lower jaw is about 30°. The distance between the tips of the lower and upper jaws (mouth gape) was

TABLE 6. Lengths (in mm) of the upper and lower jaws, width of the mouth, mouth gape when the angle between the jaws is 30°, and area (mm²) of the roof of the mouth for bluegill larvae 5 to 11 days after fertilization.

Days after fertilization	Length		Width of Mouth	Mouth gape	Area of mouth roof	Perpen- dicular from tip of lower jaw to mouth roof
	Upper jaw	Lower jaw				
5	0.23	0.14	0.25	0.13	0.06	0.14
6	0.32	0.20	0.27	0.20	0.09	0.20
7	0.44	0.43	0.32	0.23	0.14	0.43
8	0.45	0.47	0.35	0.24	0.16	0.47
9	0.54	0.51	0.26	0.27	0.14	0.51
10	0.55	0.52	0.26	0.29	0.14	0.52
11	0.65	0.52	0.26	0.33	0.17	0.52

calculated for this angle. During the feeding interval it is roughly 0.23 to 0.27 mm and reaches a maximum of 0.33 mm at 11 days. The width of the mouth appeared to decrease in size after 8 days due to the effects of starvation (Table 6).

The size of many ciliates and copepod nauplii are less than the mouth gape or mouth width. Brine shrimp nauplii (mean length = 0.47 mm) and many of the smallest adult Cladocera are too large to be accommodated. *Moina micrura*, one of the smallest Cladocera, has a mean length of 0.5 mm (Edmondson, 1959). Therefore, bluegill larvae can be expected to take only very small food organisms. Presumably, the factor limiting the size of food ingested would be the mouth gape. This increased from 0.20 to 0.27 mm from 6 to 9 days. Thus, the maximum dimension of any food item ingested during the critical period would be limited by these values.

The only food items readily distinguishable in the alimentary canals of the larvae were small unicellular algae. The red eggs and carapaces of small crustaceans were apparently in the alimentary canals of a few fish feeding in the interval 7.7 to 9.1 days. In most cases, however, unidentifiable grey-brown masses were observed in the alimentary canals of the larvae. It is likely that these masses consisted of particulate matter that was accidentally ingested as the larvae swam about with their mouths open.

The metabolism of the larvae

The energy and protein metabolism of starved bluegill larvae could give an insight into the onset and the duration of the critical period. The protein metabolism was measured to determine when protein was used as a source of food. Fat is the only other major food metabolized in fish larvae (Smith, 1957). Calculations of yolk energy and larval tissue energy could provide information on the actual sources of energy.

Energy utilization. The energy values for 16 samples of unfertilized ova ($\bar{x} = 5820 \pm 1024$ cal/g dry weight) included the amount contained in the egg membrane, and this inclusion may have introduced an error in the calculation. Assuming that the egg membrane is 1.33% of the dry weight, which was the average for the pike and carp, and that the egg membrane is largely protein (König and Grossfeld, 1913), the energy content of the egg membrane was calculated to be 7.5 cal/g dry weight on the basis that protein has a caloric value of 5650 cal/g dry weight. Since this is only 0.1% of the total initial value, it has been ignored in these and subsequent calculations.

The energy utilization of the developing embryo can be approximated by comparing the energy content of the embryo with that in the yolk sac from the time of fertilization until death. The caloric content of the yolk sac can be estimated by multiplying the caloric value of unfertilized eggs, 5820 cal/g dry weight, by the dry weight of the yolk (Table 7, Column D). Since unfertilized eggs are almost entirely yolk, very little error will be introduced by employing a caloric measurement of unfertilized eggs in lieu of measurements confined to yolk alone. Lasker (1962) showed that the nitrogen content of larval yolk and ripe ovaries of the Pacific sardine was the same. He concluded that "the similarity in quality and quantity of the major organic constituents in ripe ovarian tissue and larval yolk justifies caloric measurements of ripe ovaries be applied to larval yolk."

TABLE 7. Energy of embryos and yolk sacs between fertilization and 11 days for 1 g dry weight unfertilized ova (yolk assumed to have caloric value of 5820 cal/g dry weight and the embryo a caloric value of 5102 cal/g dry weight).

Days after fertilization	A Embryo dry weight (Table 5, Column E) (mg)	B Yolk dry weight (Table 5, Column D) (mg)	C Embryo energy (5102 cal/g dry wt. times Column A) (cal)	D Yolk energy (5820 cal/g dry wt. times Column B) (cal)	E Larval energy (Column C plus Column D) (cal)	F Day to day loss of energy (cal)
0	0	1000	0	5820	5820	390
1	81	862	413	5017	5430	368
2	164	726	837	4225	5062	156
3	324	559	1653	3253	4906	451
4	296	506	1510	2945	4455	385
5	557	211	2842	1228	4070	490
6	582	105	2969	611	3580	45
6.4	588	92	3000	533	3535	69
7	596	73	3041	425	3466	709
8	521	17	2658	99	2757	119
9	517	+	2638	+	2638	521
10	415	0	2117	0	2117	168
11	382	0	1949	0	1949	

The caloric content of 500 larvae at 11 days was determined in a calorimeter to be 5102 cal/g dry weight. Since yolk is entirely gone at 11 days, 5102 cal/g dry weight can be accepted as the caloric content of the bluegill embryo. Multiplying 5102 cal/g dry weight by the embryo weights yields the caloric values of the embryos (Table 7, Column C). The total energy of the larvae can be obtained by summing the respective energies of the embryo and its yolk sac (Table 7, Column E).

This method of estimating energy utilization is sound only if the energy content of yolk and protoplasm remain constant during development. Smith (1947) showed in the rainbow trout that the caloric value of the yolk decreased from 6246 cal/g dry weight a day after hatching to 5910 cal/g dry weight just before yolk sac absorption, a decrease of 5.4%. At the same time the energy of the embryo increased from 4270 cal/g dry weight to 5102 cal/g dry weight, an increase of 19.4%. Thus, if the bluegill is the same as the rainbow trout in these respects, the computation of energy utilization may have underestimated the caloric value of the yolk to a small extent. Furthermore, the caloric content of the embryo may be

TABLE 8. Heat production during various periods during development for an initial lg dry weight unfertilized ova.

Period in which heat is produced	Heat production	
	A Total (cal)	B Daily (cal/day)
Fertilization to hatching (0 to 2 days)	758	379
Hatching to the free- swimming stage (2 to 6.4 days)	1527	347
Free-swimming stage to 8 days	778	486
From 8 to 11 days	808	269

somewhat high during the period after hatching and before yolk sac absorption.

Larval energy loss between days (heat production) is variable as calculated in the manner described above (Table 7, Column F). There is a general tendency for heat production to rise during development, although the data are not entirely convincing on this point. If this is true, it conforms to the picture of heat production by the rainbow trout (Smith, 1947) and the Pacific sardine (Lasker, 1962). The rate of energy loss can be determined for certain developmental periods by comparing energy values between the beginning and end of the period (Table 8). The rate of energy loss in cal/day is constant from fertilization to the free-swimming stage. Energy utilization increases abruptly at that time but later decreases when starvation begins to take its toll.

Protein utilization. A small portion of nitrogen content of the eggs or larvae is in non-proteinaceous form. The non-protein part of the Pacific sardine egg is 0.7% of dry ovarian tissue (Lasker, 1962), and increases a small amount in salmonids (Hayes, 1949). Non-protein nitrogen has been disregarded in the following calculations.

Lasker (1962) showed that the nitrogen content per gram dry weight of the ripe ovarian tissue of the Pacific sardine was practically identical to that of yolk. Unfertilized ova and yolk will be assumed to contain the same percentage protein. The average nitrogen content of 34 samples of bluegill ova was $10.14\% \pm 0.45\%$ of the dry weight. Multiplying 10.14% by the factor 6.25 yielded the percentage of protein per dry weight of ova (63.37%). The protein content of the yolk sac can be calculated by multiplying the percentage protein of dry unfertilized ova (63.37%) by the weight of the yolk sac (Table 9, Column D).

The mean percentage protein of 500 bluegill embryos on day 10 and on day 11, respectively, was 71.50% of the dry weight. This is very close to the value of 70.90% for rainbow trout embryos at a comparable stage obtained by Smith (1947). Multiplying 71.50% times the weight of the

TABLE 9. Protein of embryos and yolk sacs between fertilization and 11 days for 1 g dry weight unfertilized ova (yolk assumed to have a protein content of 63.37% dry weight and the embryo a protein content of 71.50% dry weight).

Days after fertilization	A Embryo dry weight (Table 5, Column E) (mg)	B Yolk dry weight (Table 5, Column D) (mg)	C Embryo protein (71.50% times Column A) (mg)	D Yolk protein (63.37% times Column B) (mg)	E Larval protein (Column C plus Column D) (mg)
0	0	1000	0	634	634
1	81	862	58	546	604
2	164	726	117	460	577
3	324	559	232	354	586
4	296	506	212	321	533
5	557	211	398	134	532
6	582	105	416	67	483
6.4	588	92	420	58	478
7	596	73	426	46	472
8	521	17	373	11	384
9	517	+	370	+	370
10	415	0	297	0	297
11	382	0	273	0	273

embryo yields the weight of embryonic protein (Table 9, Column C). The total protein of the larvae can be obtained by summing the respective protein weight of the embryo and its yolk sac (Table 9, Column E).

The protein content of the yolk may have been overestimated to a small extent during the late stages if the protein content of the yolk decreases as it does in the rainbow trout (Smith, 1947). Smith found that the percentage protein of the yolk decreases during development, while the percentage protein of the embryo remains relatively constant. Embryo protein varied from about 73% at hatching to about 75% from 15 to 19 days later, and then decreased to about 71% when the yolk sac is absorbed.

Protein of the embryo is maximum at 7 days and then declines. Both the protein of the yolk and embryo are used between 7 and 9 days. After 9 days when the yolk sac has disappeared, the embryo continues to lose protein from its tissue. There is a precipitous drop in embryonic protein between 9 and 10 days. The larva probably encounters an energy deficit after this time which its endogenous reserves cannot make good.

Sources of endogenous energy for the larvae. The decrease in total protein for various developmental periods was compared (Table 10, Column A). These values multiplied by 4100 cal/g, the fuel value of protein when oxidized in animal tissue (Best and Taylor, 1943), yield the number of calories which has been lost in the form of protein (Table 10, Column B). Assuming that the contribution of carbohydrates is minor, subtraction of the fuel value of protein lost (Column B) from the total energy lost (Column C) will yield the caloric values of the fat oxidized during the same period (Table 10, Column D).

The relative role that protein and fat play in larval bluegill energetics (Column B and D of Table 10) varies with the stage of development.

TABLE 10. The contribution of protein and fat to the total heat production during various periods of development for 1 g dry weight unfertilized ova.

Period in which heat is produced	A Protein lost (mg)	B Heat production by protein (Column A times 4100 cal/g protein) (cal)	C Total heat production (Table 8, Column A) (cal)	D Heat production by fat (Column C minus Column B) (cal)
Fertilization to hatching (0 to 2 days)	57	234	758	524
Hatching to the free- swimming stage (2 to 6.4 days)	99	406	1527	1121
The free-swimming stage to 8 days (6.4 to 8 days)	94	385	778	393
From 8 to 11 days	111	455	808	353

Before hatching, 524 calories were contributed by fat, and only 234 calories were contributed by protein. Protein is assumed to be assimilated into tissue at this time. Between hatching and 6.4 days, fats continue to be the major source of energy; the fat: protein energy ratio is 2.8:1. However, between the free-swimming stage and 8 days, there is a marked relative increase in the contribution of energy to catabolism by protein. The fat: protein energy ratio during this period is 1:1. Fat is probably being used directly from the yolk, but after 8 days, the yolk fat has nearly disappeared and protein becomes a dominant source of energy.

DISCUSSION

The beginning of the critical period was defined as that time when bluegill larvae began to feed, and this in turn was roughly equivalent to the inception of embryonic tissue resorption. The end of the critical period is marked by the time when starvation becomes apparent in the fitness of the larvae to successfully capture food. The time when 50% of the population has perished is a direct, unbiased measure of the direct effect of starvation. The diminution of feeding success and the decrease in feeding movements are indirect manifestations of starvation. The critical period for the bluegill, according to these criteria, begins at 6.4 days and ends at 8.5 to 9 days after fertilization. Some of the criteria used above for the definition of the critical period are more useful than others for the purpose of defining the critical period. The value of each will be examined to learn if any combination of criteria can be used for the purpose of defining the critical period for fish larvae in general.

*Evaluation of attributes of starved larvae
in relation to the critical period*

Mortality. Mortality before and after yolk sac absorption in the laboratory can be attributed to a number of causes: disease, injury, environmental stresses such as low oxygen, and embryonic abnormalities resulting in a failure of normal metamorphosis at some crucial stage. The first three can be controlled in the laboratory, but the latter is difficult to separate from starvation as a cause of mortality. During metamorphosis a change from cutaneous to branchial respiration and from cutaneous to nephrogenous osmoregulation occurs. Failure of the organs to develop normally at any one of these or other stages could result in death. Some data suggest that occlusion of the alimentary canal (a failure of normal differentiation) is common in larval fishes (Vladmirov and Semenov, 1959). From this point of view, it is unfortunate that the term critical period has gained such widespread acceptance, since development of larval fish is itself a series of critical periods.

In spite of these limitations, mass mortality of unfed larvae is a good demonstration of the effects of starvation. This mortality would become apparent sometime after starvation ensued and would mark the end of the critical period. The survival curve would be ideally L-shaped, the inflection of the curve being after mass mortality had occurred. In reality, survival curves of larvae starved in the laboratory are not always of this shape. The closest approximation is the curve for very active herring larvae (Blaxter and Hempel, 1963). The choice of the inflection point on the curve for bluegill survival (or the survival of any larval species) is arbitrary. Another technique of determining the end of the critical period is the time of 50% survival, which has the advantage of eliminating subjective judgment. The curves of larval survival based on laboratory experiments are applicable only to laboratory situations. Field data may or may not exhibit a diminution in survival (Farris, 1960).

The disappearance of the yolk sac. The present data on bluegill survival and field data on survival of marine larvae presented by Farris (1960) suggest that significant mortality of starved larvae takes place before yolk sac absorption, not after yolk sac absorption as Hjort (1914) suggested. In most cases larvae begin to feed before complete yolk sac absorption: Atlantic herring (Bhattachryya, 1957); Atlantic silverside, *Menidia menidia* (Rubinoff, 1958); and cisco, *Coregonus artedii* (John and Hasler, 1956). However, Lasker (1962) reports that the jaws of the Pacific sardine are not fully formed until the yolk is gone. The bulk of the evidence favors rejecting the disappearance of the yolk sac as a primary criterion of the start of the critical period unless special developmental circumstances arise such as that mentioned by Lasker. Nor can it be equated with the end of the critical period. Although the yolk sac had disappeared by the time of 50% mortality of bluegill larvae, this is likely to be only coincidence. Blaxter and Hempel (1963) demonstrated a 4 to 5 day difference between these two events for the larvae of the Atlantic herring at 8° to 12° C. Moreover, it has been shown that endogenous energy reserves are derived from larval tissue as well as from yolk. The disappearance of the yolk sac would not indicate the degree of emaciation of the larva in view of the fact that the yolk contains only part of the endogenous reserves of energy. Therefore, disappearance of

the yolk sac cannot be used to define the beginning or the end of the critical period with unreserved confidence for all fish larvae.

Maximum length. Farris (1959) described three phases in linear growth curves of four marine larval fish kept from food. There was an initial rapid increase followed by two stages of progressive reduction in the rate of increase. The same is true for bluegill larvae, and Miller (1952) and Blaxter and Hempel (1963) reported the same for herring larvae.

Bluegill larvae reached their maximum length without food at 9 days. The time of maximum length would be a clear-cut criterion to estimate the end of the critical period, because it also closely approximates the time when feeding activity decreases, but length increase does not necessarily indicate increase in weight at this time. The data demonstrate that bluegill larvae ceased to gain weight after 7 days, two days earlier than the time when maximum length was attained.

Feeding and food seeking movements. The beginning of the critical period for bluegill larvae was arbitrarily fixed at that time when they first fed, or at about the time when they became free-swimming. This point could be established easily for other species. The incidence of food in the alimentary canal of larvae allowed to feed 24 hours after various periods of starvation has been used as an index of the feeding capacity of the larvae. As John and Hasler (1956) have pointed out, absence of food does not mean that the larvae never have fed or will never feed sometime in the future.

Feeding success of bluegill larvae may have been underestimated, because the larvae emptied their alimentary canals in a short time. Digestion in larval fish appears to take place in less than 24 hours. Bhattacharyya (1957) estimated that copepods were fully digested in herring larvae in 5.5 hours, and copepod ova were estimated to be 67% digested in 5.5 hours. John and Hasler (1956) estimated that a zooplankter passed through the alimentary canal of a larval cisco in less than 24 hours. Chiba (1961) estimated that larval carp, *Cyprinus carpio*, took 3 to 8 hours to digest meals of various numbers of brine shrimp nauplii. Morris (1955) states that larval digestion is neither fast nor complete.

Despite the possible underestimation of feeding success due to a rapid rate of digestion, the sharp decrease after 8.5 days is probably a reasonably accurate measurement of the effect of starvation. The straight-line decrease in feeding success after 8.5 days probably indicates the rate at which the effects of starvation act on the ability of the larvae to capture food. The coincidence of the change in feeding success, swimming speed, and number of rest stops after 8.5 days is strong evidence that the effects of starvation manifest themselves after 8.5 days, certainly no later than 9 days. Obviously, feeding success of larvae should be measured in defining the critical period in all larval fish.

The metabolism of starved larvae in relation to the critical period

The study of the metabolism of the larvae has made it possible to determine that the beginning of feeding was coincident with a fall in embryonic dry weight, protein, and energy. The end of the critical period

was determined by feeding success, behavior, and mortality. It is not apparent from the data whether the energy expenditure for food capture after 8 days was adequate to sustain feeding success. In general, metabolic measurements in studies on other species have not been valuable in the definition of the end of the critical period.

Resorption of embryonic tissue. Apparently, resorption of larval tissue during the period of late yolk sac absorption is not universal among all fish. Blaxter and Hempel (1963) report a decrease in embryonic dry weight of the Atlantic herring before the yolk sac disappears only for larvae hatching from large ova. But Lasker (1962) reports a loss of embryonic tissue for the Pacific sardine fry with yolk sac. Gortner (1913) and Hollet and Hayes (1946) did not show a diminution of embryonic nitrogen for the brook trout or the Atlantic salmon, respectively, but their measurements probably may not have extended far enough into the period of starvation. Smith (1947) demonstrated in rainbow trout the diminution of embryonic dry weight, protein, and energy before yolk sac absorption. The lack of uniformity among reports in the literature regarding embryonic tissue resorption before yolk sac absorption makes this phenomenon difficult to apply to the beginning of the critical period for all fish larvae, although it is apparently valid for bluegill larvae.

Substrate catabolism. Needham (1931) set forth the sequence of carbohydrate, fat, and protein in that order as the energy sources during development. If any of these foodstuffs is used exclusively at a stage of development corresponding to the critical period, chemical analysis would define the critical period. However, analysis of teleost material shows that neither Needham's sequence nor the exclusive utilization of one substrate at any time is the rule.

Hayes (1949) established the following sequence of substrate utilization for trout and salmon: fat predominantly before hatching, protein after hatching. A burst of fat usage took place midway in the post-hatching interval, followed by protein utilization until death by starvation. Smith (1952), working with the rainbow trout, states that clear peaks in the utilization of the three major foodstuffs cannot be established. In a review article, Smith (1957) brings together much information to formulate the following comprehensive description of substrate utilization by salmonoids. Protein and fat are the most important sources of energy for the developing embryo. Both non-glyceride fat (lipids) and glyceride fats are present in the yolk. Water-soluble lipids are bound to the protein in the yolk, but another fat, triglyceric acid, is found almost exclusively in the oil globule. Lipoproteins are taken directly into the embryo before hatching. Protein is utilized dominantly during hatching, but after hatching the major substrate is the water-soluble phospholipoprotein of the yolk. When the latter is largely gone, there is a burst of catabolism of the glyceride fats of the oil globule.

Just prior to the advent of the rise of glyceride fat combustion, Smith found a rise in the combustion of carbohydrates about 66 days after fertilization at the onset of what he called starvation. Carbohydrates are used during only two other phases of development, immediately after gastrulation and during hatching. It is unfortunate there are no comparable data for the bluegill, since this spurt of carbohydrate combustion seems to mark clearly the beginning of the critical period.

In the bluegill, fat was relatively more important than protein in furnishing energy to the developing embryo before hatching. Protein was utilized along with fat during the major phase of growth, but to an even greater extent after this interval. These data on substrate combustion for the bluegill appear to be of little use in defining the critical period, however. But the spurts of carbohydrate and triglyceric acid consumption discussed above are likely to have far greater utility in the estimation of the beginning of the critical period for fish larvae in general.

No single factor of those discussed, nor a single combination of them, is sufficient for defining the critical period for all fish larvae. Each investigator must choose the most fruitful criteria of those available to him. The criteria developed here for bluegill larvae do not appear to be generally applicable to all species. Probably the variable amount of yolk present in eggs and the variable speed of development of embryos of different species contribute heavily to this conclusion.

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Variations in Productivity of Goose and Hypereutrophic Sylvan Lakes, Indiana¹

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ABSTRACT

The primary productivity of a large (238.2 ha), hypereutrophic reservoir and a smaller (12.6 ha) lake of intermediate trophic level was investigated intensively for a year as part of a large program on factors controlling low productivity in marl lakes. This study together with results to be published from 11 other lakes of north-eastern Indiana embrace a broad spectrum of waters from extremely eutrophic to the infertile marl lakes and will be used as a baseline for the interpretation of physiological results of algal nutrition in the marl lakes.

Carbon-14 techniques were used to measure the *in situ* rates of primary productivity simultaneously in the three major basins of the morphometrically complex Sylvan Lake and the major depression of the relatively simple basin of Goose Lake at frequent intervals from April 1963 through March 1964. The relative significance of the productivity of the ultraplankton ($<10\mu$) to that of the larger forms was determined vertically with depth and seasonally. Productivity determinations were made concomitantly with measurements of surface and underwater light, transparency, bicarbonate alkalinity, pH, oxygen, specific conductance, ionic composition, and temperature.

Primary productivity of Sylvan Lake was correlated with incident solar radiation only in relation to general seasonal trends. Large, non-synchronous growth pulses occurred among the three major basins of the lake. Winter levels of productivity represented a significant portion, nearly a quarter, of the total annual values and was accentuated in this hypereutrophic situation where all significant photosynthesis was restricted to the uppermost meter of water. Intense self-shading effects occurred in all areas of Sylvan Lake in direct relation to the levels of surface productivity. Self-limitation of the trophogenic zone was greatest in the shallow basin receiving the major inflow, less within the blind arm of the lake, and least in the large expanse near the dam and outflow. While rates of productivity per unit volume were greater in the surface waters from inflow to outflow, the integrated values of productivity for the trophogenic zones were more uniform than would have existed without the compensating differences in self-shading effects. The estimates of primary productivity varied within a measured daily range of 9 to 4959 mg C/m²/day, with annual means of 1475, 1509, 1583, and 1691 mg C/m²/day in the progression from the inflow areas to those of the outflow. The annual mean value of 1564.4 mg C/m²/day for the entire lake represents one of the most productive lakes in the temperate region of the world.

Intrabasin variations of physico-chemical parameters of Sylvan Lake were also large and influenced considerably by morphometric variations. Vertical and seasonal gradients were great within the separate areas and fluctuated rapidly in accord with intense metabolic activities of the plankton. An account of the effects of nutrient enrichment, especially sodium, phosphate, and nitrate, of Sylvan Lake is presented with emphasis on the significance of pollutional sources from outside the immediate surroundings of the basin.

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Goose Lake, a relatively small, closed, seepage lake, exhibited intermediate levels of primary productivity in comparison to the other lakes of the region. The annual mean was 729.1 mg C/m²/day within an observed daily range of 166-1752 mg C/m²/day. Variations in productivity and physical and chemical characteristics between different areas of Goose Lake were minor. Throughout a large period of summer stratification the major portion of the productivity occurred in the metalimnion where several oxygen maxima developed. The major metalimnetic oxygen maximum was neither so strongly developed nor of the duration that has been observed in many other lakes. The situation in Goose Lake likely represents a transitional stage in the trophic development of lakes in which metalimnetic oxygen maxima occur, and it is discussed in relation to the difficulties encountered in attempts at maxima and lake classification.

The morphometry, high relative depth, and protection afforded by the surrounding terrain of Goose Lake, coupled with unusual meteorological conditions during 1963-1964, permitted a condition of temporary meromixis to develop. Circulation was complete following the loss of ice cover in 1964. The conditions under which such a temporary biogenic meromixis can occur is discussed in view of the belief that similar situations are common among small, deep, and protected lakes of the glaciated Midwest.

TABLE OF CONTENTS

	Page
Introduction	148
Methods	149
Sylvan Lake	151
Description of the lake	151
Primary productivity of Sylvan Lake	154
Physico-chemical characteristics and intrabasin variations	159
Goose Lake	168
Description of the lake	168
Primary productivity of Goose Lake	170
Physico-chemical characteristics of Goose Lake	173
Discussion	176
Sylvan Lake	176
Goose Lake	180
Acknowledgments	181
References	182

INTRODUCTION

The glaciated region of northern Indiana contains approximately 1000 lakes greater than two hectares in size. About 15% of these waters are included in the general category of marl lakes characterized by high alkalinity, high pH values, and marked deposits of calcareous materials. Productivity at all levels of organisms is moderate to extremely low, and the marl lakes represent those closest to what one may consider oligotrophic that occur in Indiana.

An intensive investigation was initiated in 1962 on the causal relationships between low algal productivity in marl lakes and environmental parameters, with particular emphasis on inorganic and organic nutritional factors. The studies on algal nutrition and factors controlling growth in marl lakes are reported elsewhere (Wetzel 1965a, 1966, in preparation). Essential to the physiological investigations, however, is an accurate appraisal of the productivity of the marl lakes relative to others of the region. Such information is sparse for the area under consideration and

essentially non-existent for the productivity of the microorganisms. Therefore a broad spectrum of 13 lakes was selected for determination of the annual rates of primary productivity and relevant physico-chemical characteristics. The productivity of a majority of the lakes falls into the general realm of mesotrophic, some of which are not marl lakes. Goose Lake in the present analysis is typical in this respect. At the most productive end of the spectrum of lakes is Sylvan Lake, an old hypereutrophic reservoir. The rate of algal photosynthesis and related physico-chemical factors were investigated for one complete year.

Primary productivity has been used as an index of the potential productivity of a body of water in many diverse ecosystems in various parts of the world. The literature in this area, which is voluminous, has been reviewed at great length by Vinberg (1960), Wetzel (1964a), and Fogg (1965), among others. The techniques for assaying primary productivity have been refined considerably and represent some of the few methods of moderate sensitivity that are available for direct *in situ* determinations of rates of growth. While the techniques permit a considerable degree of accuracy, many detailed questions remain concerning physiological variables among different methods and their interpretation.

Regardless of whether the autotrophic source of organic matter is utilized indirectly, following bacterial alteration, or directly by higher trophic levels, rates of photosynthetic growth represent an excellent measure of the maximum that is possible under the integrated interaction of the multiplicity of dynamic environmental factors regulating growth. To be sure, allochthonous, chemosynthetic and other heterotrophic sources of non-photosynthetic energy input occur, the magnitude of which is largely unknown. Preliminary investigations (*e.g.*, Kusnetzow, 1959; Sorokin, 1964; Wright and Hobbie, 1965; Wetzel, 1967) indicate that these sources are significant but generally far subsidiary to autotrophic productivity.

METHODS

Determinations of the primary productivity of the phytoplankton were made employing *in situ* carbon-14 methodology, although the productivity of Sylvan and Goose lakes is of sufficient magnitude during most of the year for application of the less involved "light-and-dark" bottle oxygen techniques. The two methods are not directly comparable, however, without several assumptions, most of which may be variable (*cf.* Strickland, 1960). Moreover, in that the productivity analyses of these lakes are part of a larger comparative investigation of marl lakes of Indiana, some of which are at or below the sensitivity of oxygen techniques, it was desirable to apply uniform techniques to all the lakes under consideration.

Details of carbon-14 techniques have been described elsewhere (Doty and Oguri, 1959; Strickland, 1960; Goldman, 1963; Wetzel, 1964a), all with minor modifications from their original introduction by Steemann Nielsen (1951, 1952). Recapitulation will be brief here with emphasis on a few modifications employed in the present work.

Samples of water were collected at close depth-intervals (0.5-2 m, depending on the lake and the thickness of the trophogenic zone) with an opaque, non-metallic water sampler (Van Dorn, 1956). The samples were placed immediately into scrubbed, thoroughly rinsed, ground-glass-stoppered, clear and opaque Pyrex bottles (125 ml). An automatic syringe was used to inject rapidly a milliliter of sterile C-14 as sodium bicarbonate of known assay into each sample. Samples were sealed immediately and suspended from an anchored buoy at the depth from which they had been collected. Care was exerted to avoid shading effects of the buoy. Effects of wave action on the buoy-suspension system no doubt helped to maintain the plankton in suspension, and hence it is doubtful that settling of a majority of the plankton was significant during the brief incubation period. Samples were placed into a light-free specially-constructed box after an incubation period of 4-5 hours (never exceeding 6 hours). This period is adequate for significant uptake of C-14 and yet sufficiently brief to minimize bottle effects (Vollenweider and Nauwerck, 1961). As soon as possible, aliquots were filtered through filter membranes having a porosity of $0.45 \pm 0.02\mu$ (HA, Millipore Filter Corp., Bedford, Mass.). Simultaneously, productivity of plankton of a larger size was fractionated by filtration through membranes with a porosity of $10.0 \pm 3.0\mu$ (Millipore OS). Vacuum pressures were never allowed to exceed 38 cm Hg and generally were in the range of 13-26 cm Hg. Air was not permitted to pass through the filters after filtration.

After desiccation, filters were exposed to fumes of HCl for a brief period for removal of C-14 precipitated or complexed as monocarbonates (Wetzel, 1965b). Activity of the filters was determined with a gas-flow Geiger-Muller counter with a micromil window (D-47, Nuclear-Chicago Corp.). The efficiency of the solid counter was repeatedly determined by standards of known absolute activity. Absolute activity was determined again by combustion to gas-phase, detection by ionization chamber procedures, and comparison to standard sources. Calibration of the counter was routinely checked throughout the periods of assay.

Radiocarbon used in the field was prepared in large, uniform lots, quickly ampouled, and sterilized. Random samples were chosen from the lots and assayed by combustion and analysis in gas-phase by ionization-chamber techniques. Similar gas-phase techniques have been employed in phytoplanktonic productivity studies by Miyake *et al.* (1954), Goldman (1960), Goldman and Wetzel (1963), and Wetzel (1964a), and in periphytic and macrophytic analyses of productivity (Wetzel, 1963, 1964a, 1964b).

Computations were based on a graphic integration by planimetry of the net activity per square meter of water column. Non-photosynthetic carbon fixation, absorption, cosmic and instrument background radiation are compensated for in all calculations by the integrated areal subtraction of the radioactivity of plankton of dark bottles from those of the light. An isotope effect of 6% was used in the computations (Steemann Nielsen, 1952, 1955; Sorokin, 1959). Total inorganic carbon available for photosynthesis was determined from total alkalinity values, pH, and temperature with the convenient table of Bachmann (Saunders *et al.*, 1962). The productivity of the incubation periods was expanded to the total daily productivity value of the phytoplankton separately for each

day from the total photoperiod of daily pyrhelimeter curves. The incubation periods were computed by planimetry as a fraction of the integrated total area of the insolation curve. This method compares favorably with diurnal measurements of phytoplankton productivity at 4-hour intervals and integration of the increments.

Samples were collected at bi-weekly intervals during the more productive months of the year (April through September) and at monthly intervals during the remainder of the year.

Intensity of surface solar radiation was measured with a recording pyrhelimeter (Instruments Corporation, Baltimore, Maryland) from which light ($\text{Ly/day} = \text{g.cal/cm}^2/\text{day}$) was integrated by planimetry. Precision of recording is within $0.1 \text{ g.cal/cm}^2/\text{minute}$, with a maximal time lag in response of two minutes. Light values are expressed as that portion of the light in the photosynthetic range (380-760 $\text{m}\mu$) by the factor (0.5) of Strickland (1958:467). This factor varies somewhat with atmospheric conditions and height of the sun but is realistic for a majority of the conditions. Underwater light intensities were determined with a Weston photocell unit (Whitney Instruments, Inc., San Luis Obispo, California). Profiles of water temperature were determined with an electrical-resistance thermistor thermometer (Yellow Springs Instruments, Yellow Springs, Ohio) calibrated against a corrected thermometer of the National Bureau of Standards. All thermal values are expressed in degrees Celsius ($^{\circ}\text{C}$).

Alkalinity and oxygen concentrations were measured at frequent depth-intervals, following the procedures of Standard Methods (American Public Health Association *et al.*, 1960). The pH values were determined electrometrically (Beckman Model N-2 pH meter). Techniques of Standard Methods were similarly employed for ionic analyses. Analyses of concentrations of minor elements were determined spectrochemically (Kopp and Kroner, 1966). Percentage of dissolved-oxygen-saturation was determined with enlarged nomograms of Mortimer (1956). A direct reading conductivity meter, automatically referenced to 25°C (Industrial Instruments Inc., Cedar Grove, N. J.) was used for *in situ* measurements of specific conductance.

SYLVAN LAKE

Description of the lake

The shallow trough of the present basin of Sylvan Lake is largely the result of the meandering pattern of the north branch of the Elkhart River. This river originates from numerous small tributaries to the south-east of the lake and meanders northwestward along the northern portion of the present lake basin (Fig. 1). Flow continues in a southerly direction to the main branch of the Elkhart River. The latter river again swings to the north, joins the St. Joseph River, and eventually flows through southwestern Michigan to Lake Michigan.

In 1836 the Legislature of Indiana authorized the construction of a navigable feeder canal from the present lake area to a point of intersection of a major ship canal, to extend across the northern part of Indiana for



Fig. 1. Hydrographic map of Sylvan Lake, Noble County, Indiana. See text for details regarding sampling stations and delineation of areas of the basins. Redrawn and altered from the original map of W. M. Tucker, 1925. Elevation 279.3 m (average lake level elevation, 1943-1953, Perry and Corbett, 1956).

the proposed Michigan and Erie Canal, with the Elkhart River (Owen, c. 1930). The rapid development of railroads in the region resulted in a complete abandonment of the partially completed canal system at a total financial loss around 1840. The feeder dam, however, was completed in 1839, forming Sylvan Lake of approximately its present size and volume. The dam ruptured several times: in 1839, before completion; in 1844; and again in 1855. The decaying dam structure was again nearly lost under flood conditions in the spring of 1877. The dam tumbles were relocated slightly in 1892 and replaced with a concrete dam in 1904, the present structure retaining the lake.

The basin of Sylvan Lake is very shallow with the exception of two small depressions in the southeast arm (Fig. 1). Sampling stations were selected to incorporate the three major areas of the lake: *A*, the large shallow area near the outlet (locally known as "Lower Spread"); *B*, the blind arm containing two relatively deep depressions ("Cain Lake"); and *C*, the shallow northeastern arm ("Gravel Pit Lake") that receives the major inflow from Henderson Creek, formerly part of the north branch of the Elkhart River. A fourth area, *D*, ("Upper Spread"), is circumscribed from the above areas in Figure 1 by lines *x-x*, *y-y*, and *z-z*. Although these areas were delineated arbitrarily for the productivity calculations, they are sufficiently variable in their biological and physical characteristics to warrant such a separation. Pertinent morphometric data are given in Table 1.

The immediate area of Noble County surrounding Sylvan Lake is included in the Maumee Lacustrine section of the Northern Moraine and Lake region of Indiana (Logan *et al.*, 1922). The region was greatly modified by Late Wisconsin glaciation; morainic glacial till and outwash deposits dominate the area. Glaciofluvial outwash plains and moderate terracing are typical of the terrain surrounding Sylvan Lake and the Elkhart River system. Nearly all the soil immediately surrounding the lake is poorly drained, of very gentle slope, of the Carlisle-muck type of high organic matter, and unsuited for cultivation (Rogers *et al.*, 1953). Isolated areas of Fox and Bellefontaine sandy loams occur in a variegated distribution along the shoreline as well as in the immediate vicinity. A large deposit of gravel is located along the north side of the northeastern arm (Station *C*, Fig. 1). Cultivation is intense in a large area to the north of the lake. Sylvan Lake is completely surrounded by cottages and homes and is used primarily for recreational purposes.

TABLE 1. Morphometric data of Sylvan Lake, Noble County, Indiana. See text for explanation of areas *A*, *B*, *C*, and *D*.

	Total	Area A	Area B	Area C	Area D
Area (ha)	238.2	67.6	44.3	60.6	65.7
Volume (m ³ X 10 ⁶)	7.53	2.85	1.54	1.30	1.84
Maximum depth (m)	11.0	7.3	11.0	4.2	5.8
Mean depth (m)	3.19	4.2	3.5	2.1	2.8
Length of shoreline (m)	22,783				
Shoreline development	4.16				
Relative depth (<i>Z_r</i>) (%)	0.63				

Primary productivity of Sylvan Lake

The photosynthetic plankton productivity of the different areas of Sylvan Lake was variable and extremely great (Fig. 2) and fluctuated markedly on an annual basis. Only general similarities were found among the three separate areas. A spring peak in productivity was evident during spring circulation immediately after ice breakup. High levels of productivity occurred throughout the summer, with violent fluctuations of short duration. A majority of these population growth-pulses was non-synchronous among the three major basins of the lake, produced by relatively independent phytoplankton populations. Although primary productivity was reduced considerably under heavy ice cover, it represented a very significant portion of the total annual productivity. The productivity of the period from 1 November 1963 through 15 March 1964 constituted 11.5, 22.3, and 17.4% of the total annual productivity at Stations A, B, and C, respectively. The daily mean values for this same period were considerable: Station A, 194.7; B, 336.3; and C, 257.2 mg C/m²/day. Under the more restrictive winter conditions the rates of growth were more uniform than during other periods of the year.

The phytoplankton productivity was severely restricted to the upper layers at all times. Intense self-shading effects occurred at all stations and varied directly with the magnitude of surface productivity. Station C, representing the shallow, most productive inflow area of the lake, was most severely affected in this respect. Productivity values were extremely high in this location, and practically all the significant fixation occurred in the first one-half meter (Fig. 3). In the blind arm of Sylvan Lake, represented by Station B, the self-shading effects were considerably reduced. The depth of light penetration was greater and, although variable, the trophogenic zone extended to greater depths, generally 1-2 m. Photosynthetic productivity in the large area near the dam and outlet, represented by Station A, was considerably reduced, and self-shading effects were of less significance. The trophogenic zone extended to depths of several (3 to 4) meters in this case. The trophogenic zone was also severely limited to the very surface waters under ice cover.

The intense restrictions of the photic zone and rapid extinction of light was demonstrated markedly by the variations in underwater light penetration among the basins (Fig. 4). The depths of the 1% level of surface radiation ranged from 2-7 m at Station A, 1-3 m at Station B, and 1-2 m at Station C. The increasing extinction coefficients (k) for visible radiation and estimates of relative transparency further emphasized the reduction of the photic zone progressively from Stations A to B to C (Table 2). Inorganic turbidity was not a significant factor in these differences. Light is unquestionably a dominant environmental factor controlling growth in such a hypereutrophic situation. Seasonal variations in surface solar radiation (Fig. 5) are reflected only very generally in the annual fluctuations in primary productivity as given in Figure 2. Of greater significance are the self-limiting effects of high population densities in regulating the thickness of the photic, and hence the trophogenic, zone in the different basins of the lake.

The primary productivities of the different basins of Sylvan Lake were highly variable and distinct on a volumetric basis. Rates of growth

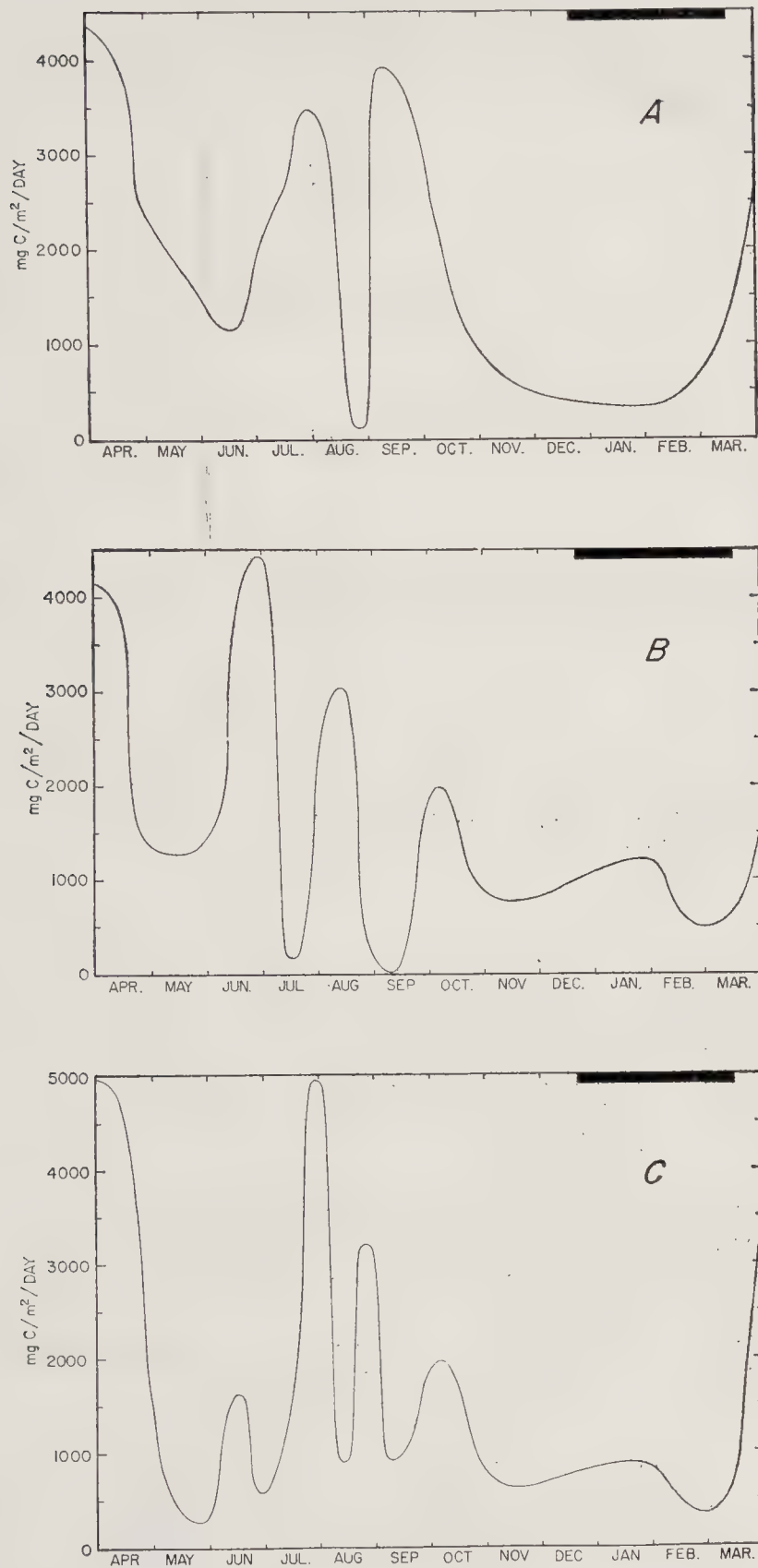


FIG. 2. Seasonal and intrabasin variations in primary production rates at Stations A, B, and C of Sylvan Lake, 1963-1964, expressed as integrated mg C synthesized per m² water column per day. Opaqued area = ice cover.

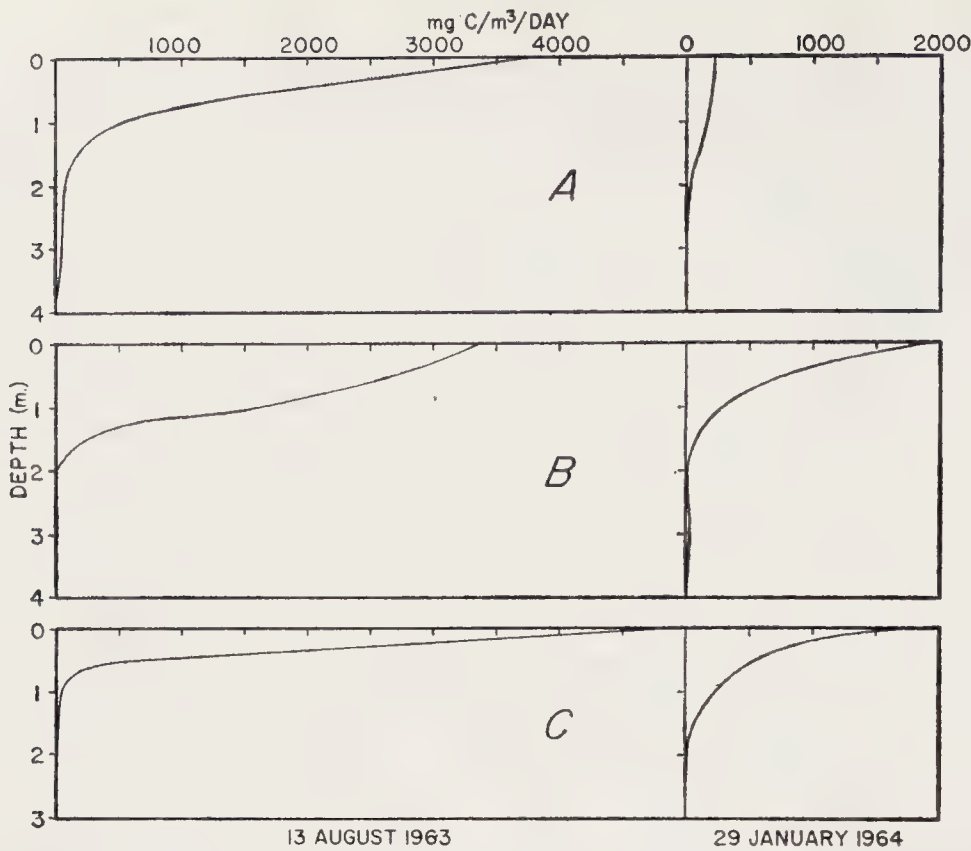


FIG. 3. Vertical variations in the depth of the trophogenic zone in the three basins of Sylvan Lake.

in the surface cubic meter of water were approximately 3-4 times greater in Area C than in A, and about 2 times greater in Area B than in A. Seasonal variations in this relationship are many, however, and large deviations occurred when the population rates of growth were out of phase among the different basins. Such discrepancies were particularly true of the rates of productivity in Area B, which is relatively isolated from the other areas of the lake.

The mean daily productivities on an annual basis and the ranges of daily primary productivity in Sylvan Lake are given in Table 3 for the different areas from April 1963 through March 1964. Productivity of Area D, circumscribed by the other areas (lines *x-x*, *y-y*, and *z-z* in Fig.

TABLE 2. Extinction coefficients (*k*) of visible radiation and relative transparency (Secchi) at Stations A, B, and C, Sylvan Lake, 1963-1964.

Station	Mean <i>k</i>	Range of <i>k</i>	Mean transparency (m)	Range of transparency (m)
A	2.95	1.58—4.5	0.90	0.41—3.65
B	3.19	1.05—4.5	0.76	0.30—2.30
C	3.41	1.85—4.5	0.56	0.30—0.85

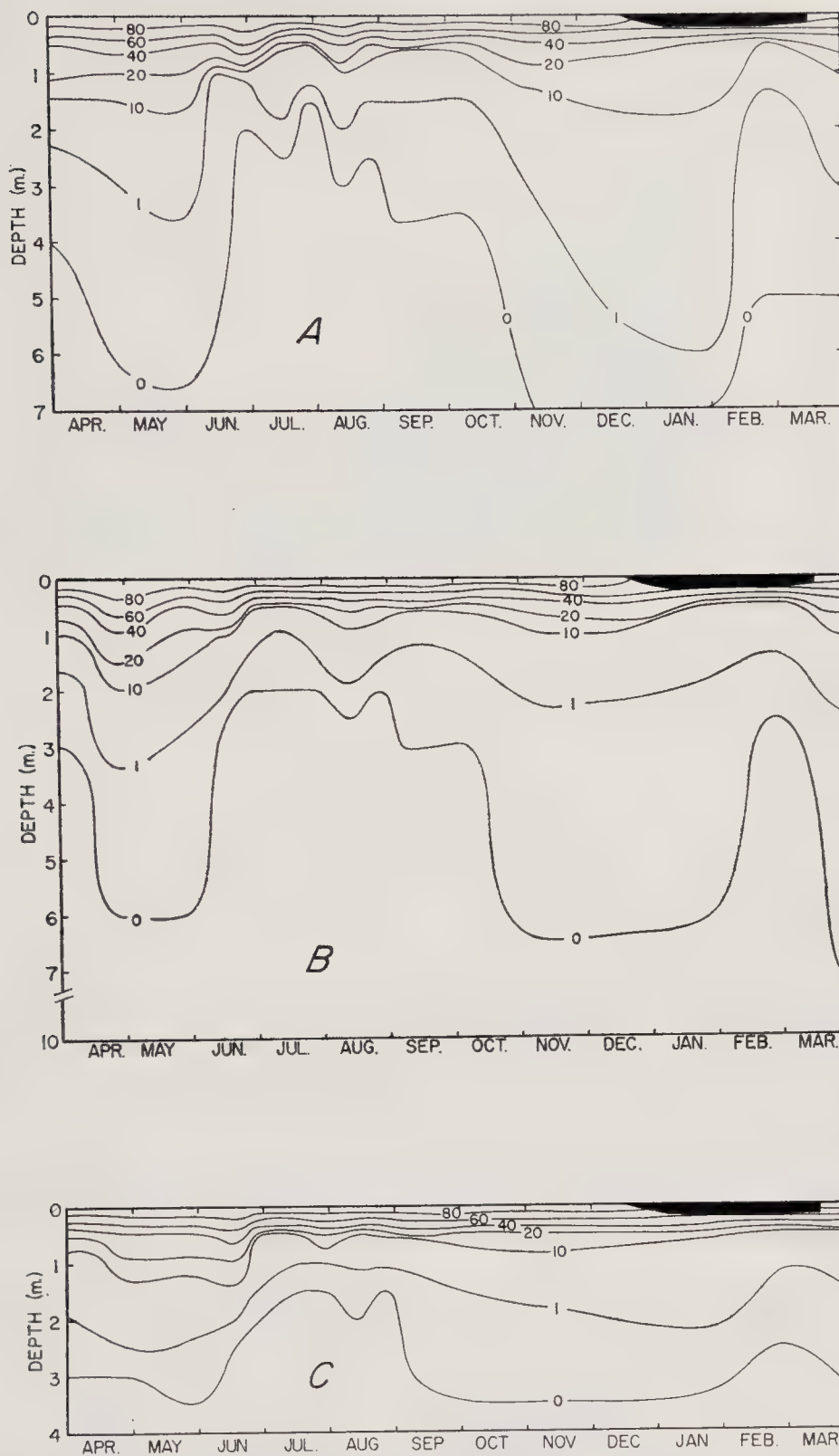


FIG. 4. Isopleths of percentage penetration of surface light underwater among the basins at Stations A, B, and C of Sylvan Lake, 1963-1964. Opaqued area = ice cover.

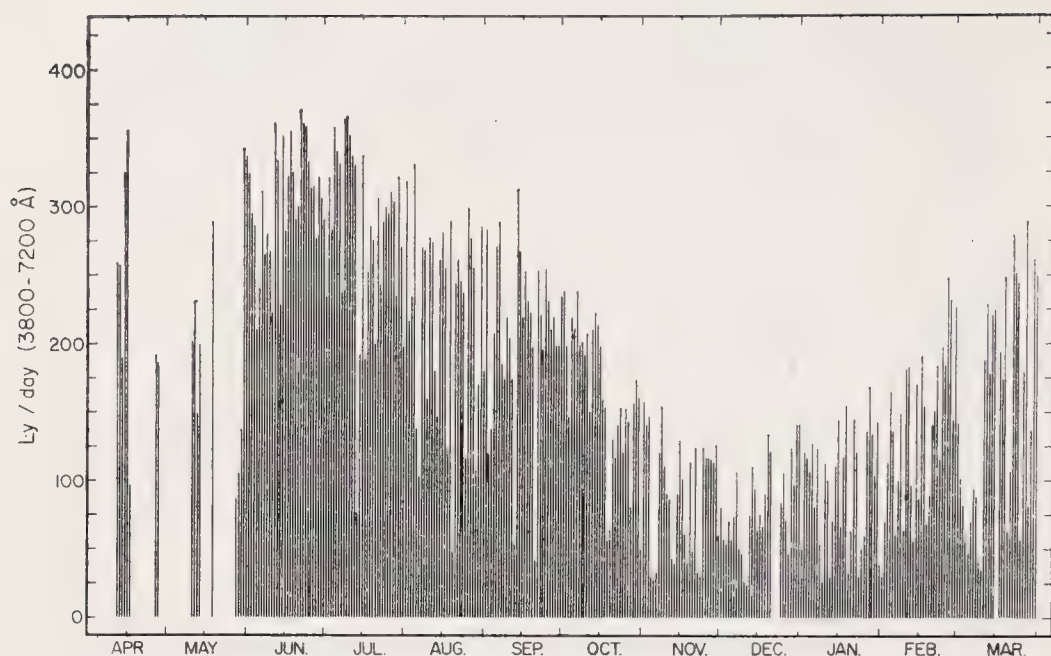


FIG. 5. Annual surface light intensity (Langleys/day = g-cal/cm²/day) at 380-720m μ . Gaps represent periods where no data were available and periods when the instrument raft was inaccessible due to unsafe ice conditions.

1) was calculated as the mean of the productivities of areas *C* and *A*. The mean did not have to be weighted, because the areas of basins *A* and *C* are similar. While the estimate of the productivity of Area *D* must be viewed as approximate, this region of the lake is very likely a transition zone between the shallow inflow area and the larger expanse near the outflow. The contribution of Area *B* to the central area is relatively minor.

The estimates of the annual mean daily values of primary productivity are extremely large, making Sylvan Lake one of the most productive bodies of fresh water yet recorded (*cf.* Table 10 of Wetzel, 1964a; Wetzel, 1966; Vinberg, 1960; Westlake, 1963). The only higher values known to the author are from a small, highly enriched Danish lake, Pedersborg Sø, with an annual productivity of 664 g C/m² (Johnsen *et al.*, 1962) and a highly polluted German lake (W. Ohle, personal communication); that

TABLE 3. Mean daily productivities on an annual basis and ranges of daily photosynthetic productivity in Sylvan Lake, Indiana, for the period April 1963 through March 1964. Values of areas *A*, *B*, and *C*, observed; area *D*, calculated (see text).

Area	Mean		Range (mg C/m ² /day)	Annual productivity (kg C/ha)
	(mg C/m ² /day)	(mg glucose m ² /day)		
<i>A</i>	1690.8	4227	109-4115	6171
<i>B</i>	1509.2	3773	9-4438	5509
<i>C</i>	1474.8	3687	258-4959	5383
<i>D</i>	1582.8	3957	—	5777
Entire lake	1564.4	3911	9-4959	5710

of Sylvan Lake is 571 g C/m^2 for the entire lake and 617 g C/m^2 at Station A. Only in highly modified fresh waters, such as sewage lagoons, have growth rates been found to exceed the maximal daily values of nearly $5 \text{ g C/m}^2/\text{day}$ that occurred in Sylvan Lake. The high sustained values, even under ice cover, of primary synthesis of organic matter is reflected in other trophic levels of the lake. Growth of the fish populations is extremely rapid and is among the fastest recorded in continental waters of this latitude (S. D. Gerking, personal communication).

Physico-chemical characteristics and intrabasin variations

Thermal variations within the complex basin of Sylvan Lake are major and directly related to large morphometric differences. A large portion of the basin is of sufficient depth and volume to permit typical dimictic circulation (areas represented by Stations A and B in Fig. 6). Thermal stratification occurred earlier, however, in the deeper areas and persisted for a considerably longer period, with much greater thermal density gradients than in the more shallow areas. The blind arm of Area B is somewhat more protected by the surrounding terrain than other areas of the lake and is less subject to the effects of wind action. Summer thermal stratification is very temporary in the large shallow areas (Station C, Fig. 6; also most of Area D) and occurs only during intermittent periods of very warm, calm weather. Surface temperatures of nearly 30°C are common, which are higher than most neighboring waters of similar fetch, undoubtedly due to absorption of heat by high biogenic turbidity.

Thickening of the epilimnion occurred rapidly in the deeper areas in late summer, followed by a progressive cooling to temperatures slightly below 4°C just prior to formation of ice cover. Inverse stratification was also variable among the basins. Heating was more rapid in the shallow areas, probably as a result of marginal heating through the ice and from the sediments. In February and early March a series of mild periods occurred, causing a marked change in the ice condition. Melt water, accentuated by rain water passing through the porous ice, altered the stratification profiles considerably (Fig. 6). Ice breakup occurred rapidly on 17 March, followed by a cold period during which the water was again cooled well below 4°C .

The extremely high planktonic productivity of the upper layers of Sylvan Lake and intense bacterial metabolism, coupled with varying degrees of thermal stratification, is reflected in several of the annual distributional patterns of chemical parameters. Very high values of alkalinity, often exceeding 7.6 me/l , were rapidly attained in areas A and especially B where depth is sufficient for sustained stratification (Fig. 7). Biogenic decalcification occurred commonly in the epilimnetic waters in all three areas of the lake. However, seasonal variations were large among the different basins, and this was particularly true of Area C with weak and persistently interrupted stratification.

The upper surfaces values of pH (Fig. 8) must be viewed as approximate, since considerable diurnal photosynthetically-induced fluctuation occurred. While such diurnal variations are not significant (less than 0.1 unit) in most waters of low to moderate productivity (Wetzel, unpublished data), in the hypereutrophic situation here fluctuations were moderately

large, 1-2 units per day. The values reported here represented mid-morning determinations. The pH of the hypolimnetic areas is more stable and not so subject to the rapid changes of the surface layers. The acidic conditions of the hypolimnia of the basins again emphasize that these are situations of intense bacterial metabolism. Concentrations of hydrogen sulphide increased rapidly as stratification developed to within three meters of the surface. High pH values in the surface strata and similarly a rapid increase in alkalinity in the lower layers occurred beneath ice cover as a direct result of the relatively high rates of productivity during winter.

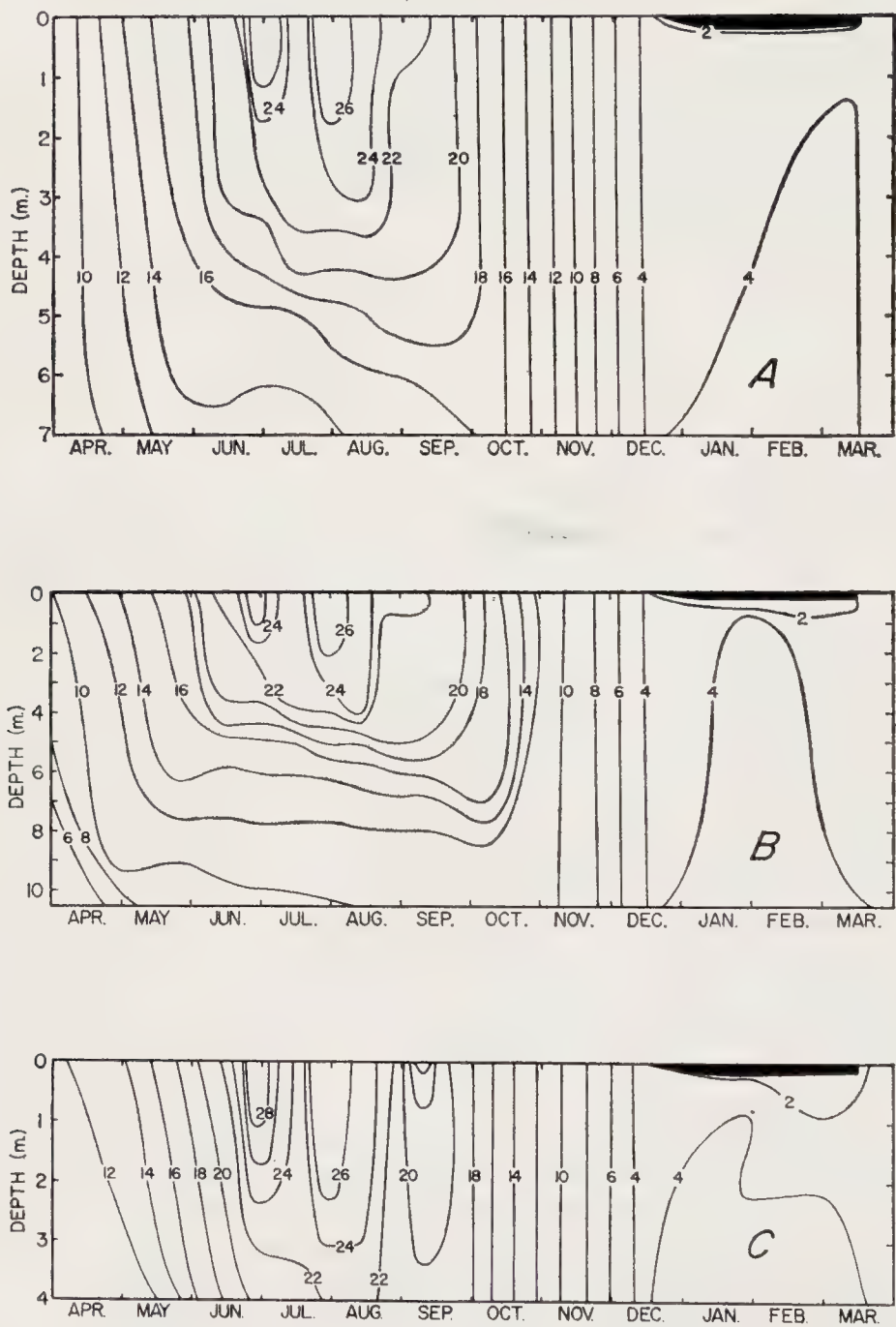


FIG. 6. Isothermal variations ($^{\circ}\text{C}$) in the three major areas (Stations A, B, and C) of Sylvan Lake, 1963-1964. Opaqued area = ice cover.

Concentrations of dissolved oxygen were extremely high in the surface layers and rapidly depleted in the hypolimnia of the basins (Fig. 9), as would be expected in such a productive situation. The determinations of epilimnetic oxygen concentrations are mid-morning values as were those of pH and are subject to similar diurnal fluctuations. Depletion of oxygen occurred very early in the warmer period of the year. Anaerobic conditions persisted well into the autumn, and for a major part of the year a large portion of the volume of the lake was completely anaerobic. Percentage saturation values were extremely high, and often supersaturation

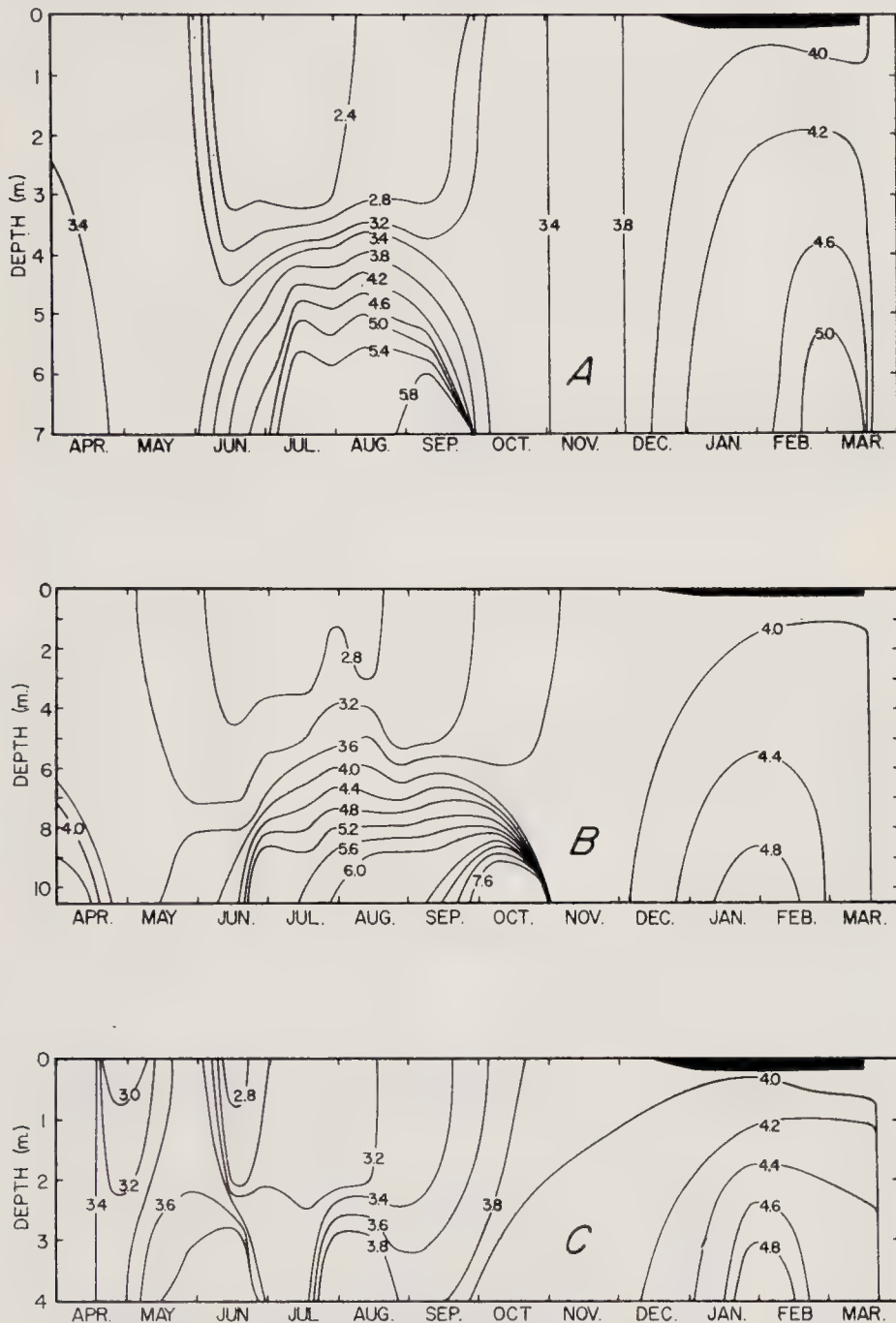


FIG. 7. Isopleths of alkalinity in milliequivalents per liter of the three major areas (Stations A, B, and C) of Sylvan Lake, 1963-1964. Opaqued area = ice cover.

approached 300% at the surface on calm days (Fig. 10). Entrapped and accumulated oxygen beneath ice cover reached very high concentrations (24.5 mg/l, 185%) in February. Supersaturation was so great that, upon puncturing the ice, the water literally effervesced with the release of oxygen. Hypolimnetic depletion and anaerobic conditions were produced again in the terminal phases of the winter period.

The high productivity of Sylvan Lake is influenced by many factors. The morphometry of the basins is such that much of the lake is subject to frequent circulation and regeneration of nutrients. Enrichment by polluttional activities of man also contributes to the high levels of productivity. The lake is almost completely surrounded by 700 homes and

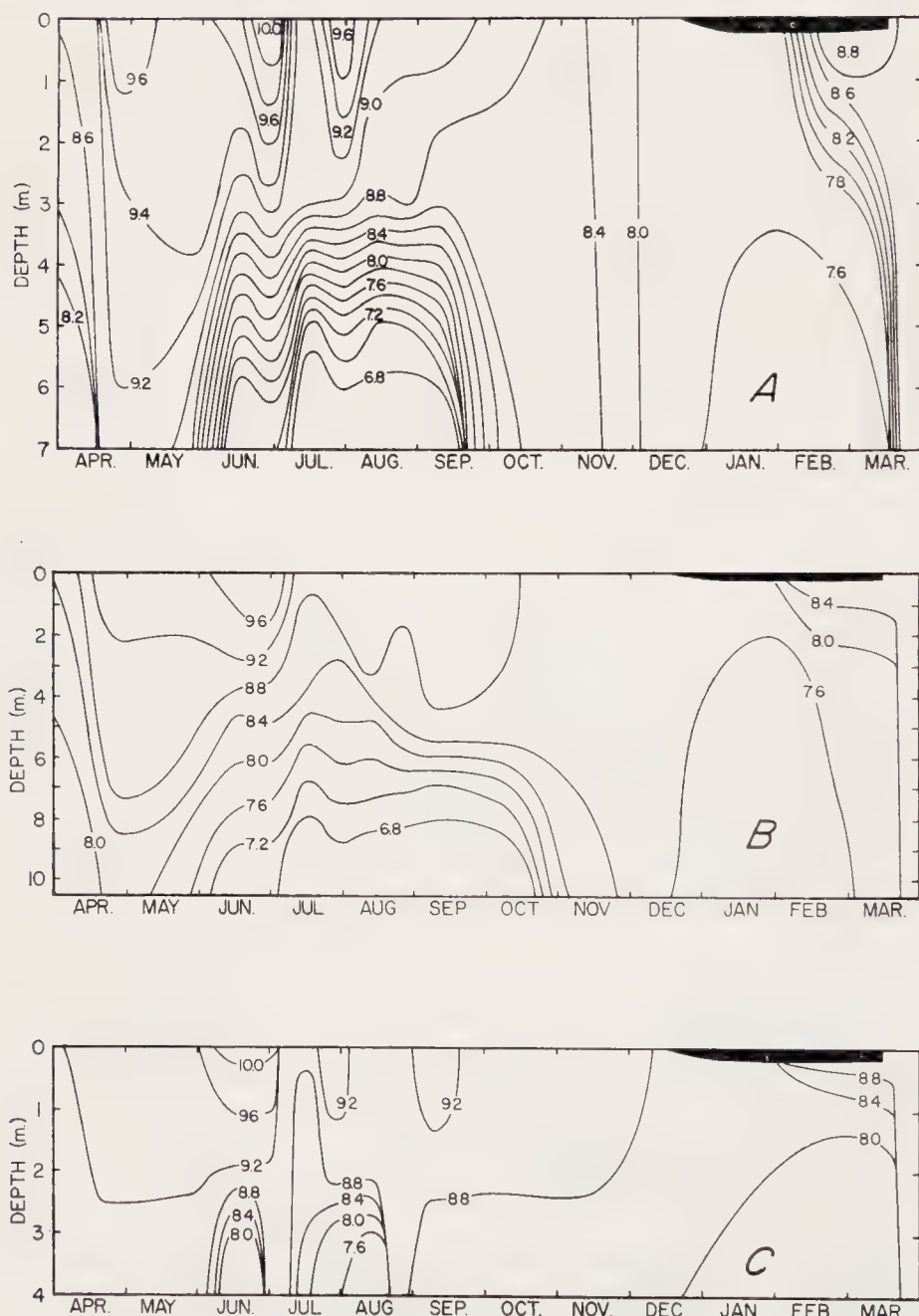


FIG. 8. Isopleths of pH of the three major areas (Stations A, B, and C) of Sylvan Lake, 1963-1964. Opaqued area = ice cover.

cottages. Various quantities of sewage, both treated and untreated, are received by the lake. The major inflow occurs via Henderson Lake Ditch from the south directly into Gravel Pit Basin Area C (Fig. 11). This creek has a relatively sluggish flow except during periods of heavy rainfall and runoff. Large amounts of effluent are received for a stream of its volume. Most of the effluent originates from the vicinity of the city of Kendallville approximately 7 km to the southeast of Sylvan Lake. Total phosphate and nitrate concentrations are extremely high in the areas of sewage outfall in the surroundings of Kendallville (Table 4). Concentrations from small contributing lakes are relatively low and of minor

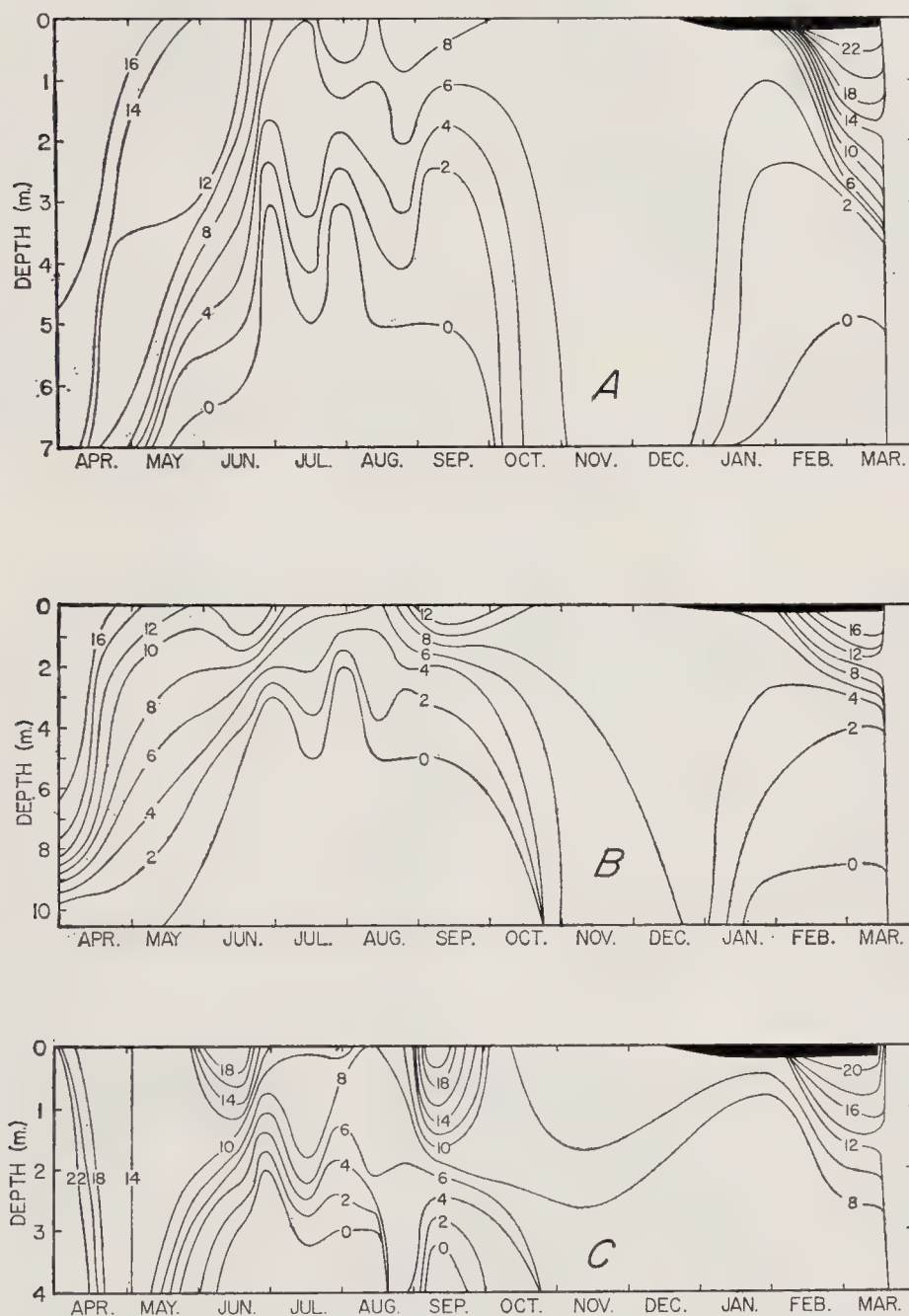


FIG. 9. Isopleths of concentrations of dissolved oxygen in milligrams per liter at Stations A, B, and C of Sylvan Lake, 1963-1964. Opaqued area = ice cover.

significance. The concentrations of phosphates and nitrate are reduced rapidly to 1-3 mg/l, however, in progression towards Sylvan Lake. Within Sylvan the concentrations are often reduced further due primarily to biogenic utilization. Algal concentrations increase to extremely high numbers immediately below the outfall of Henderson Lake and the effluent of the sewage disposal plant (S. D. of Fig. 11) of Kendallville (Table 5). As the stream progresses to Sylvan Lake, the algal numbers are reduced rapidly to the lesser concentrations of the lake *per se*. An overwhelming dominance of bluegreen algae occurred throughout most of the year in all basins of Sylvan Lake, particularly *Anabaena flos-aquae* (Lyngbye)

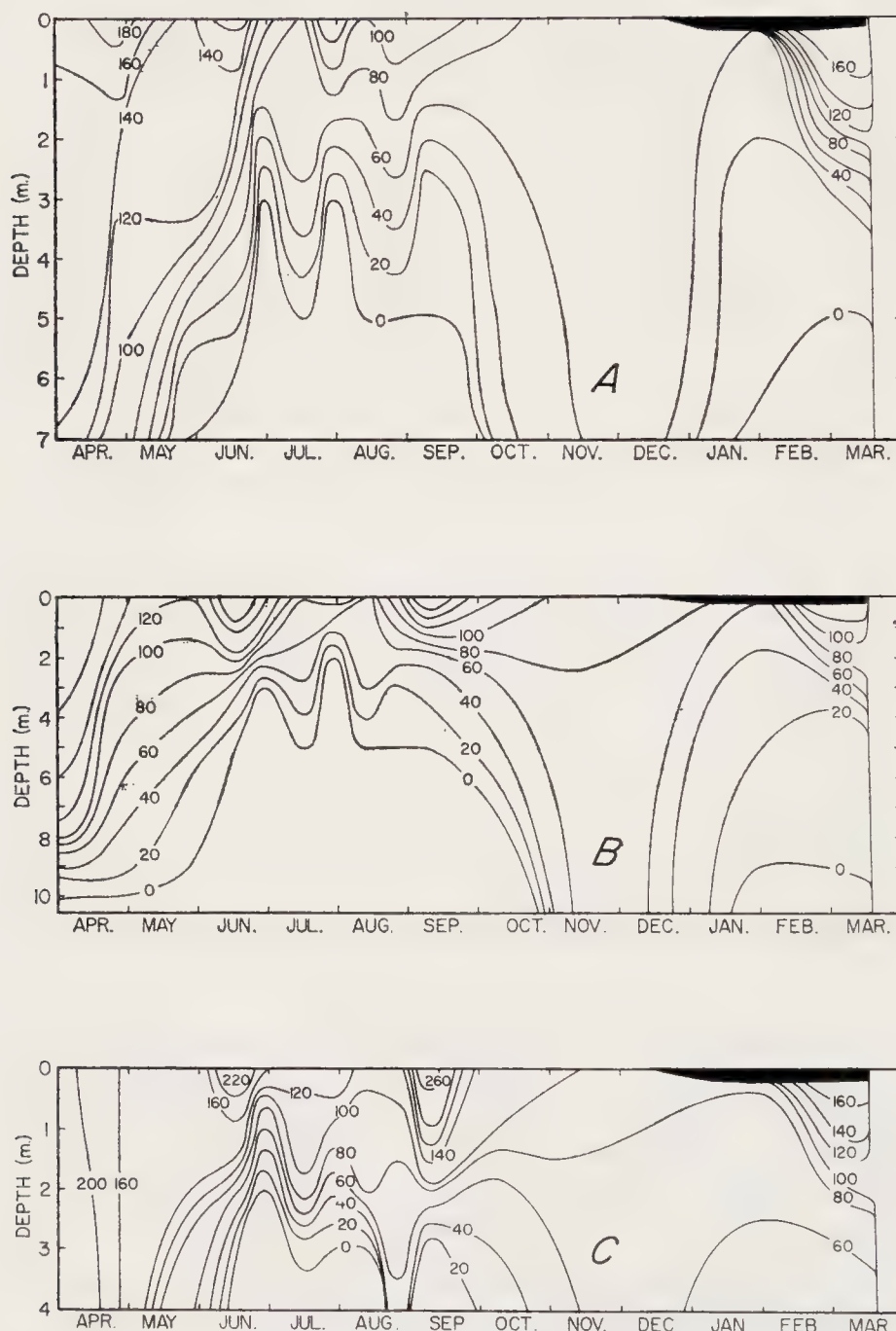


FIG. 10. Isopleths of percentage saturation of dissolved oxygen at Stations A, B, and C in Sylvan Lake, 1963-1964. Opaqued area = ice cover.

TABLE 4. Water analyses in the tributary flow from the Kendallville region to Sylvan Lake, Noble County, Indiana. Station locations correspond to those of Figure 11. From unpublished data of John Winters, Indiana State Board of Health.

Dates	Station	Total PO ₄ (mg/l)	NO ₃ (mg/l)	B.O.D (ppm)	Turbidity (ppm)
7 Sept. 1961	4	23.0	0.4	4.8	30
	5	14.0	1.6	10.0	15
	6	12.0	3.7	7.0	10
	10	3.8	0.4	11.0	10
	12	6.3	3.0	2.4	5
	14	4.5	1.0	4.6	5
	16	5.6	0.1	6.2	25
	18	6.0	0.1	20.0	10
	20	5.0	<0.05	—	—
11 July 1962	1	0.04			
	1a	0.4			
	2	0.35			
	3	6.2			
	3a	8.0			
	4	2.9			
	5	20.0			
	6	5.0			
	8	0.1			
	9	0.05			
	11	2.4			
	12	2.0			
	13	0.05			
	15	0.05			
	20	0.8			
15 Aug. 1962	4	1.8			
	5	16.0			
	6	4.5			
	8	0.1			
	9	0.05			
	11	3.2			
	12	2.6			
	13	0.05			
	15	0.08			
15 April 1963	4	0.05			
	6	5.0			
	12	2.6			
	20	1.0			
3 July 1963	4	0.9			
	5	14.0			
	7	6.8			
	12	4.0			
	19	0.2			

Brebisson, *Anacystis*, and *Euglena*. Effects of pollutional influences have been noted frequently in the literature; a recent example of similar effects on a chain of lakes immediately northeast of Sylvan Lake has been briefly investigated by Dean (1964).

TABLE 5. Algal concentrations and dominant forms of inflow system to Sylvan Lake, Noble County, Indiana, 3 July 1963. Station locations correspond to those of Figure 11. From unpublished data of John Winters, Indiana State Board of Health.

Station	Concentration (millions of cells liter)	Dominant forms (in decreasing abundance)
4	2.58	<i>Diatoma</i> , <i>Navicula</i> , <i>Cyclotella</i> , <i>Euglena</i> , <i>Scenedesmus</i> , <i>Gomphonema</i> , <i>Synedra</i> , <i>Ulothrix</i>
5	47.44	<i>Scenedesmus</i> , <i>Chlorella</i> , <i>Euglena</i> , <i>Ankistrodesmus</i> , <i>Closterium</i> , <i>Cyclotella</i>
7	92.08	<i>Scenedesmus</i> , <i>Chlorella</i> , <i>Euglena</i> , <i>Ankistrodesmus</i> , <i>Closterium</i> , <i>Oscillatoria</i>
12	26.60	<i>Scenedesmus</i> , <i>Chlorella</i> , <i>Euglena</i> , <i>Diatoma</i> , <i>Microcystis</i> , <i>Cyclotella</i> , <i>Gomphonema</i> , <i>Cylindrospermum</i> , <i>Ankistrodesmus</i> , <i>Closterium</i>
17	14.80	<i>Anabaena</i> , <i>Cyclotella</i> , <i>Oscillatoria</i> , <i>Spirogyra</i>

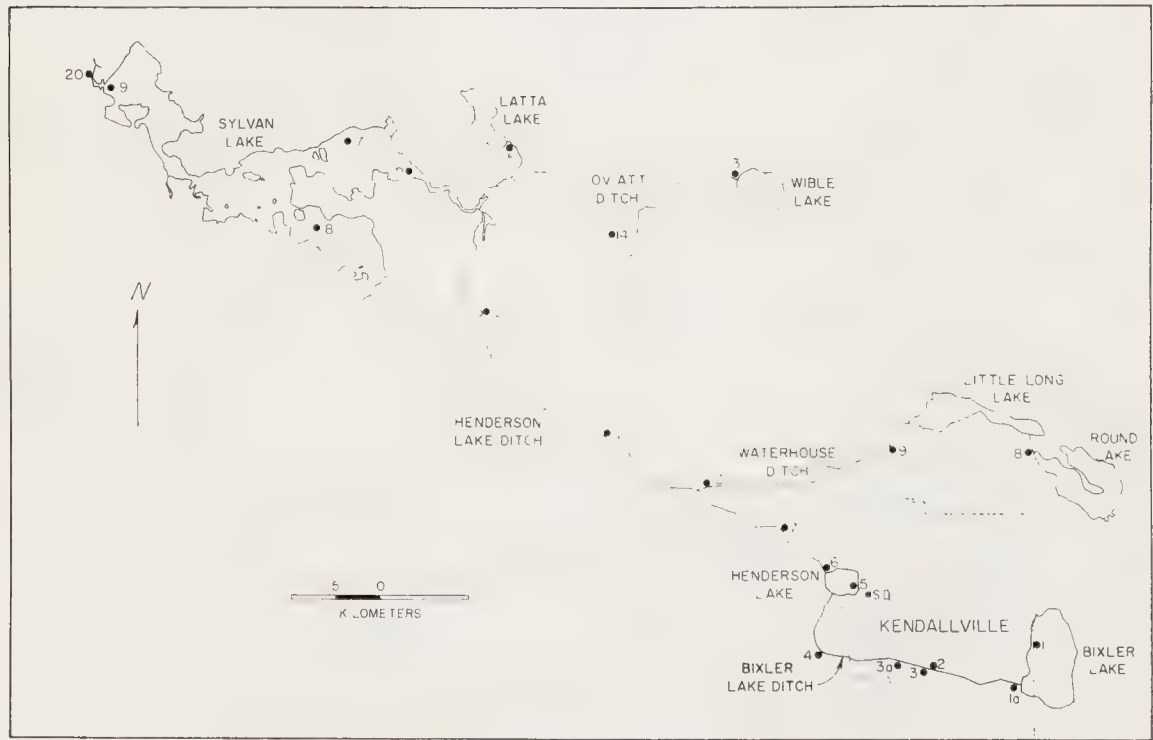


FIG. 11. Diagrammatic map of the primary inflow sources of Sylvan Lake. Sampling stations correspond to those of Tables 4 and 5. S.D. = sewage disposal plant of Kendallville.

TABLE 6. Ionic analyses of surface waters of Stations A, B, and C, Sylvan Lake, Noble County, Indiana. Values are given in mg/L.

Date	Depth (m)	Station	Ca ⁺⁺	Mg ⁺⁺	K ⁺	Na ⁺	Cl ⁻	SO ₄ ⁼	Total PO ₄ ⁼	NO ₃ ⁻	Mn ⁺⁺	Fe ⁺⁺⁺
29 July 1963	3	A	38	18	4.0	52	48	52	—	—	—	—
5 Oct. 1963	3	A	51	18	3.5	38	55	48	1.6	—	—	—
29 Jan. 1964	3	A	69	22	3.0	48	68	44	3.5	—	—	—
22 Mar. 1964	1	A	66	20	3.0	47	61	45	1.8	0.2	<0.05	0.08
29 July 1963	5	B	46	17	4.0	50	47	48	—	—	—	—
5 Oct. 1963	5	B	50	19	3.5	38	56	46	2.8	—	—	—
29 Jan. 1964	5	B	67	21	3.0	46	65	25	3.7	—	—	—
22 Mar. 1964	1	B	63	21	3.0	44	57	42	1.0	0.2	<0.05	0.06
29 July 1963	1	C	51	18	4.0	55	59	52	—	—	—	—
5 Oct. 1963	1	C	57	20	3.5	45	66	48	2.9	—	—	—
29 Jan. 1964	1	C	69	21	3.0	46	65	45	3.1	—	—	—
22 Mar. 1964	1	C	68	19	3.0	54	72	47	1.5	0.2	<0.05	0.10

The salinity of Sylvan Lake is high, and the lake water would be classified as hard (Hutchinson, 1957). Cationic proportions (Table 6) deviate somewhat from that of typical lakes of open river systems, a result of rather high sodium concentrations particularly at the inflow Area C. The order fluctuates seasonally between calcium and sodium, but a tendency exists towards $\text{Ca} > \text{Na} > \text{Mg} > \text{K}$. Anionic proportions also fluctuate seasonally with a general order of $\text{CO}_3 > \text{Cl} > \text{SO}_4$, typical of hard-water lakes. The relatively high sodium and chloride concentrations may be derived from several origins, although there is again a suggestion that these ions are derived largely from polluttional sources, *e.g.*, Na from detergents. The values of the isolated arm, Area B, of the lake are somewhat lower and relatively independent of the main flow from areas C to A. Enrichment by drainage from agricultural areas was not deemed significant in comparison to other sources of nutrients. Many other lakes in the immediate surrounding watershed are much more typical in their degree of trophic development than Sylvan Lake and receive volumetrically similar runoff from cultivated areas. Goose Lake, described in detail below, may be viewed as typical of many of the lakes of this region. These data, as well as the analyses of productivity, other parameters, and dominant algal forms, all indicate a highly enriched situation in Sylvan Lake.

Comparison of the relative importance of the ultraplankton ($<10\mu$) to larger forms in the total autotrophic productivity demonstrate the major significance of larger plankters in Sylvan Lake. Seasonal shifts in the significance of the ultraplankton occurred with highest percentage values in the winter and spring periods. The smaller forms accounted for a small proportion of the total production rate, with a maximal range of 0 to 72.2%. These values are considerably lower than the autotrophic productivity by the ultraplankton fraction in less productive environments in the surrounding area. The discussion and hypothesis proposed by Goldman and Wetzel (1963) concerning the adaptive significance of decreased plankton size in more restrictive environments is in general confirmed by the results from Sylvan Lake and 12 other lakes of northern Indiana. These findings will be discussed comparatively and detailed elsewhere (Wetzel 1966, in preparation).

GOOSE LAKE

Description of the lake

Goose Lake lies approximately 58 km to the southwest of Sylvan Lake in the center of Kosciusko County, 4 km south of the city of Warsaw. The depression of Goose Lake occurs in a shallow valley of a moderately rolling outwash plain of interlobate morainal deposits of the late Wisconsin glaciation. The deep hole of the lake basin *per se* is probably a result of the deposition of a large mass of ice with the formation of a typical kettle depression. The remainder of the lake, primarily the southeast end (Fig. 12), is likely a result of irregularities in the ground moraine. Many of the lakes of the region were probably formed in this fashion by shallow discontinuities in ground moraine (Scott, 1916), with deep regular depressions the result of typical kettle blocks of ice.

Goose Lake is essentially a closed, seepage lake with an encompassing watershed of about 5 km². An inlet on the north side and an outlet on the south side of the shallow end of the lake (north and south, respectively, of Station *B*, Fig. 12) are at best intermittent. A large area to the west of Station *A* is swampy and densely forested. Annual water fluctuations are relatively minor, less than 0.3 m, and were not considered in the calculations of morphometric data (Table 7).

The majority of the watershed and the land surrounding the lake consists of soil of Miami loam, rolling phase (Tharp *et al.*, 1927). The soil is relatively poor in organic matter, phosphorus, and nitrogen, and does not lend itself to intensive cultivation. Agriculture is generally marginal in the area. The basin is well protected by the surrounding terrain, much of which consists of forested, steep hills. Much of the area about the lake is uninhabited, and pollution to any significant extent is not evident at the present time.

The littoral region of Goose Lake is generally narrow. Relief is gradual for only a few meters from the shoreline, after which the slope descends precipitously to depths of 6-8 m. The gradient at depths beyond 8 m is less. Biogenic and inorganic turbidity is not great except during peaks of phytoplankton pulses. Light attenuation occurs rapidly, however, within 4-8 m of depth. As a consequence of these factors, macrophyte

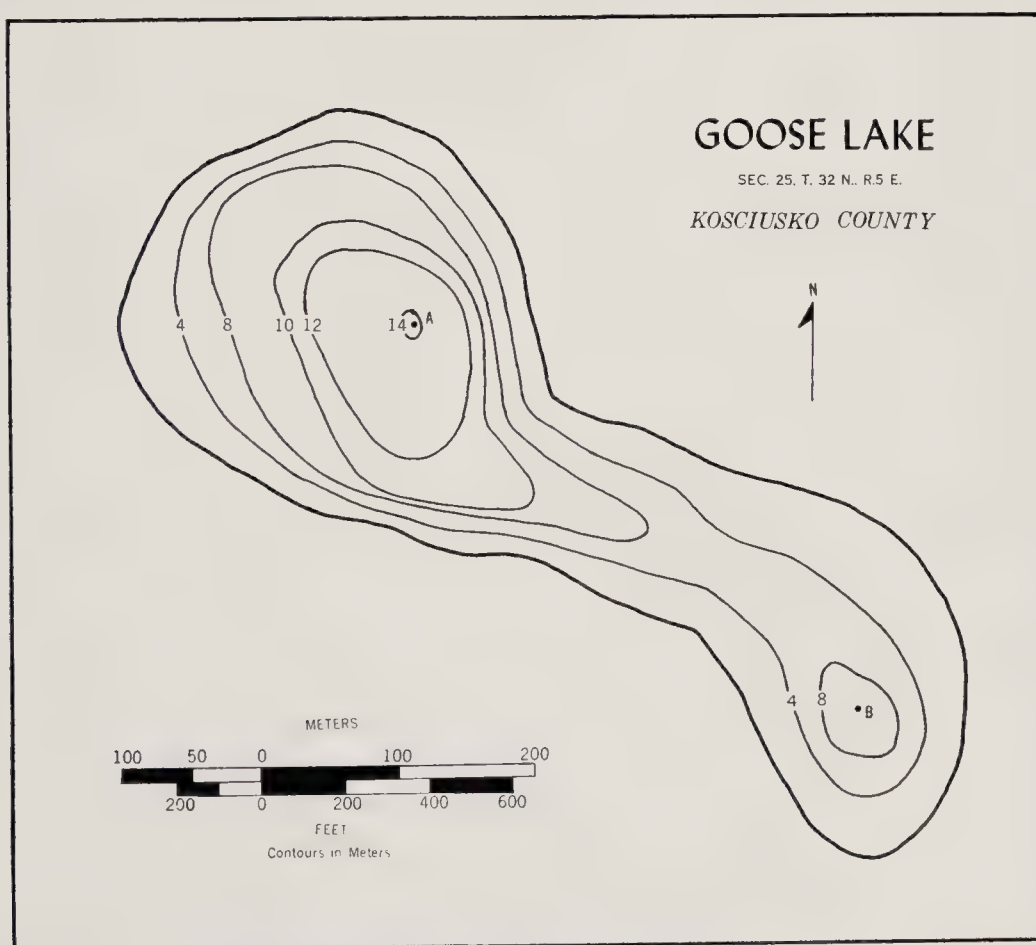


FIG. 12. Hydrographic map of Goose Lake, Kosciusko County, Indiana showing the positions of Stations *A* and *B*. Redrawn and modified from original map of Will Scott (?), date unknown. Elevation 342 m.

TABLE 7. Morphometric data of Goose Lake, Kosciusko County, Indiana.

Area (ha)	12.56
Volume ($\text{m}^3 \times 10^6$)	0.764
Maximum depth (m)	14.0
Mean depth (m)	6.08
Length of shoreline (m)	1809
Shoreline development	1.44
Relative depth (Z_r) (%)	3.55

distribution is limited largely to the shallow littoral area. Conspicuous representatives include: *Ceratophyllum demersum* L. and *Elodea canadensis* Michx., common on slopes; occasional *Sagittaria*; and rarely three species of *Potamogeton*.

Primary productivity of Goose Lake

The integrated photosynthetic productivity of the phytoplankton of Goose Lake exhibited relatively sustained, moderate levels throughout the warmer periods of the year (Fig. 13). Small peaks in rates of growth occurred in April and in late summer before productivity decreased markedly during the initial months of the winter period. Shortly before the ice cover left the lakes a very significant rise in productivity was found. The seasonal productivity curve followed the trend of incident solar radiation (Fig. 5) in a general way but with many periods of deviation.

The daily mean primary productivity of Goose Lake over the year was 729.1 mg C/m²/day, within an observed range of 166.4 (10 May 1963) to 1752.0 (21 June 1963) mg C/m²/day for the period April 1963 through March 1964. These estimates represent a daily mean of 1.82, and a range of 0.42-4.38, g glucose/m²/day on an equivalent basis. The productivity of the period from 1 November 1963 through 15 March 1964 constituted 24.3% of the total annual productivity. The daily mean value for this same period was 177.1 mg C/m²/day. In assessing the degree of trophic development of Goose Lake in comparison to other lakes (*cf.* Table 10, Wetzel 1964a, 1966, and unpublished data), these data are upper intermediate and fall into the very general, and ambiguous, category of an upper mesotrophic lake.

The vertical distribution of productivity demonstrated marked seasonal variations. A few examples selected from the annual series to point out some of the observed variations are given in Figure 14. The profile of 10 May 1963 was typical during the spring and fall periods of relatively low productivity. The productivity curves of 4 June (Fig. 15), 28 June, and 9 August 1963 represent some of the variations that developed during periods of oxygen maxima in the metalimnion. The bimodal vertical distribution of 21 June 1963 occurred frequently but usually was more accentuated in the metalimnion. Under ice cover the trophogenic zone was considerably more restricted to the surface, as one would expect, than during ice-free periods, as noted in the productivity curves of 31 January and 28 February 1964. During the period of dense

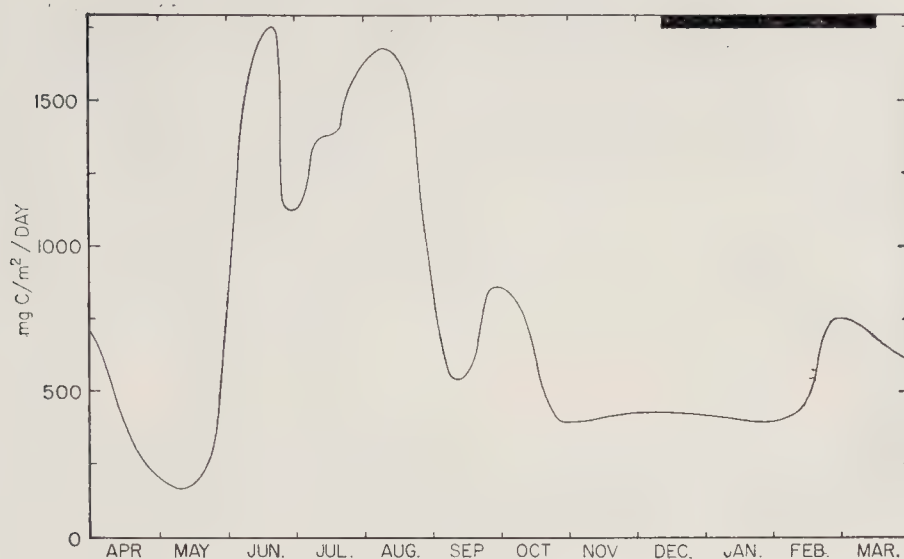


FIG. 13. Primary production rates at Station A, Goose Lake, 1963-1964, expressed as the integrated mg C per m² of water column per day. Opaqued area = ice cover.

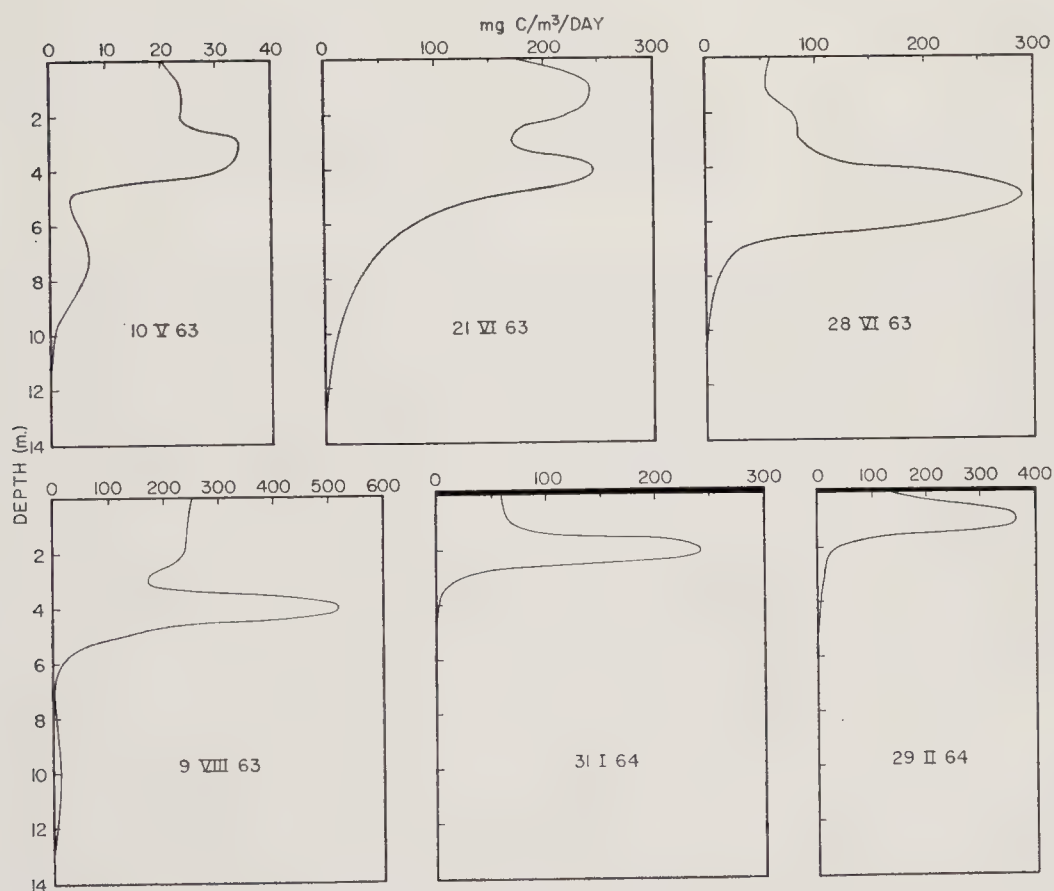


FIG. 14. Examples of seasonal variations in the vertical distribution of primary production rates, Station A, Goose Lake. Opaqued areas = ice cover to scale. Note large differences in the productivity scales.

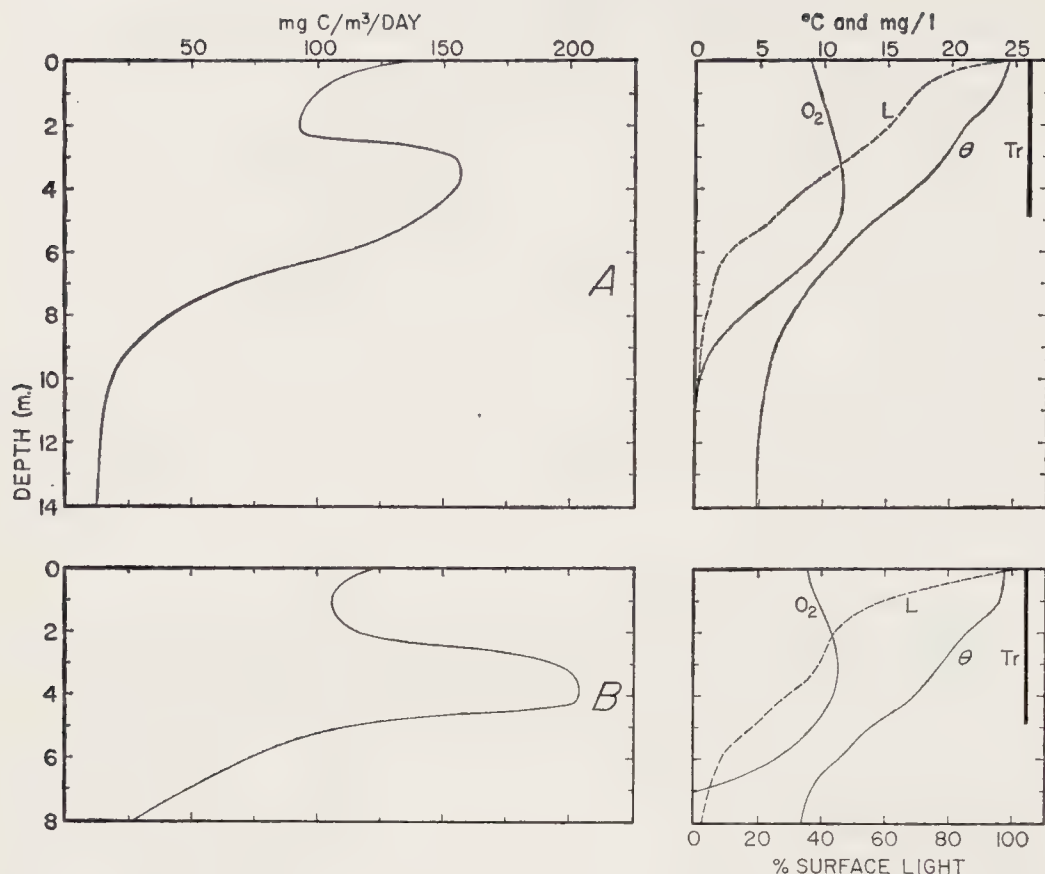


FIG. 15. Variations in primary production rates, temperature (θ), oxygen, percentage penetration of surface light (L), and transparency (Tr) between Stations A and B, Goose Lake, 4 June 1963.

populations of plankton in the metalimnion, under-water light was rapidly attenuated with increasing depth (Fig. 16). This was especially true during the week of 21 June 1963. The vertical extinction coefficients (k , visible radiation) were roughly typical of lakes of intermediate productivity, with an annual mean of 0.91 within a range of 0.37–1.65. Estimates of transparency (Secchi disk depth) ranged from 1.0 to 6.4 m, with an annual mean of 3.8 m. Severe surface inhibition of growth was noted at all times of the year except during periods of heavy cloud cover.

During the peak of the primary metalimnetic oxygen maximum, the dominant phytoplankton of the metalimnion were *Ceratium hirundinella* (O. F. Muell.) Dujardin and *Oscillatoria agardhii* Gomant. Less common representatives of the metalimnion and epilimnion at this time included *Fragilaria*, *Tabellaria*, *Dinobryon*, *Staurostrum*, and *Anabaena*.

Variations in primary productivity and physical and chemical parameters between Stations A and B were not great (Fig. 15). The productivity curve of the shallow area, B, had the same configuration as that of the deep station although somewhat more compressed. The integrated productivity of Station A to the same depth as that of B was 1135.9 mg C/m²/day. The comparable volume of water at Station B resulted in an integrated value of 1087.0 mg C/m²/day, in very close agreement to that of Station A. Differences in oxygen concentrations, temperature, light distribution, and transparency were very slight. Marked deviations occurred only immediate to the bottom of Station B.

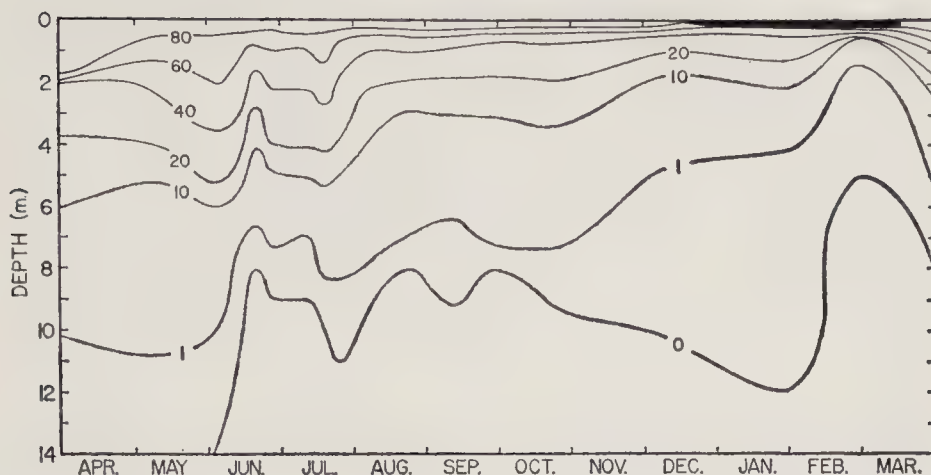


FIG. 16. Isopleths of percentage penetration of surface light underwater, Station A, Goose Lake, 1963-1964. Opaqued area = ice cover.

The ultraplankton ($<10\mu$) of Goose Lake were quantitatively of less importance than is commonly the case in many of the lakes of the region. The fraction of the total productivity accomplished by the ultraplankton ranged from 0 to 42.5%. Distinct vertical shifts in size were evident, with the smaller forms largely restricted to the epilimnion. During the periods of metalimnetic oxygen maxima, the larger forms completely dominated the metalimnion. As a consequence, the productivity was weighted greatly by the plankton larger than 10μ during most of the stratified periods of the year.

Physico-chemical characteristics of Goose Lake

The physico-chemical characteristics of Goose Lake during the period of investigation strongly suggest a circulatory pattern of partial meromixis. Although relatively shallow with a maximum depth of 14 m, the surface area of the lake is small and well protected from the effects of wind action. Isothermal conditions at 4° or less were not found (Fig. 17). Following the very severe winter of 1962-1963, circulation in the temporary monimolimnion was not significant below 10 m. Spring and summer thermal stratification set in very rapidly soon after the ice left the lake on 29 March 1963. Limits of the metalimnion developed rapidly at the 4-8 m layer and persisted until late November. During the vernal period the dissolved oxygen concentrations remained well below 2 mg/l and 20% saturation at a depth of 10 m (Fig. 18, 19). The incomplete spring circulation was further demonstrated by the distribution of alkalinity (Fig. 20) and hydrogen-ion concentration (Fig. 21).

In late November, however, circulation was more nearly complete than in the spring but again did not penetrate entirely into the deepest layers. Circulation occurred in the deep water, bringing the oxygen levels up to 6.3 mg/l and 50% saturation at 12 m by early December. Specific conductance rose considerably below the first two meters in December, and then remained fairly consistent at 260 μ mho (at 25°C) for the remainder of the profile. During an extremely cold period in mid-December, the lake froze very rapidly on the 16th and 17th. Perhaps the 6° isotherm curve of Figure 17 should be depressed somewhat more

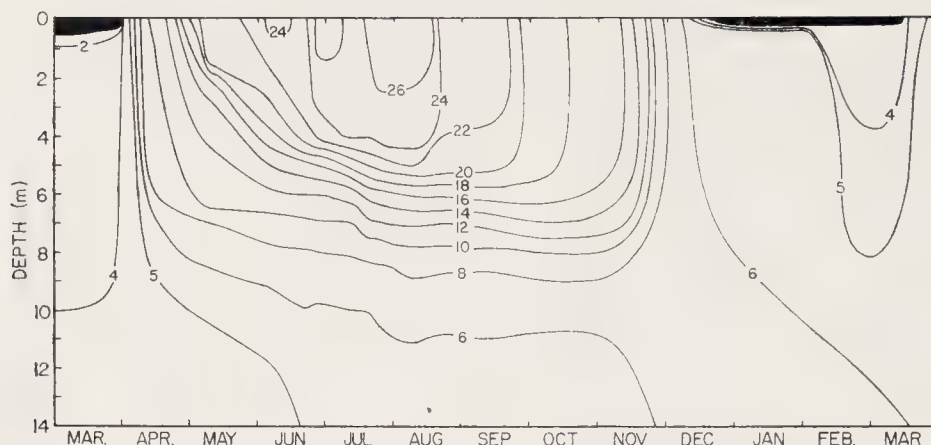


FIG. 17. Isothermal variations ($^{\circ}\text{C}$) at Station A, Goose Lake, 1963-1964. Opaqued area = ice cover.

just before ice formation, as data are lacking for several days prior to this time. Nonetheless, it is doubtful that complete circulation occurred. By the end of January the 5° isotherm remained just beneath the ice and the 6° isotherm at 10.4 m. Conductivity increased markedly between 0 and 1 m, from 152 to 243 μmho , and further increased progressively to 270 μmho at 12 m. The lower waters were again being depleted of oxygen at this time (Fig. 18, 19).

February and early March 1964 exhibited marked fluctuations between periods of cold and of very mild conditions with heavy rainfall, during which the ice became porous and permeated with air spaces. The rainfall and large open areas in the littoral region likely permitted considerable marginal inflow, allowing a depression of the colder water to deeper areas. Water of 6° was still found near the very bottom but was soon dissipated. Oxygen deficit and alkalinity increased in the lowermost layers below 10 m. Ice cover was lost rapidly on 14-15 March, during a warm, windy period. At this time complete circulation occurred in a typical fashion for most of the lakes of the area.

A metalimnetic oxygen maximum appeared very early in the season in May during a spring peak of very high productivity. Levels of 12.8

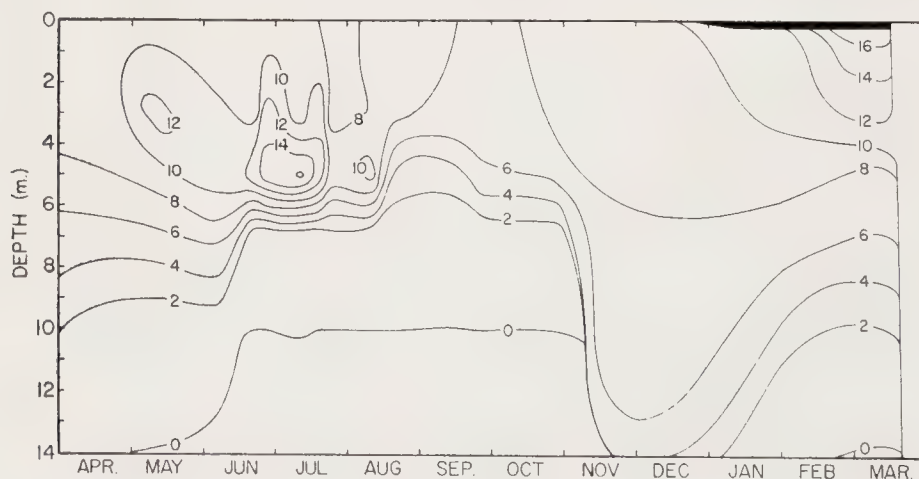


FIG. 18. Isopleths of dissolved oxygen concentrations in milligrams per liter, Station A, Goose Lake, 1963-1964. Opaqued area = ice cover.

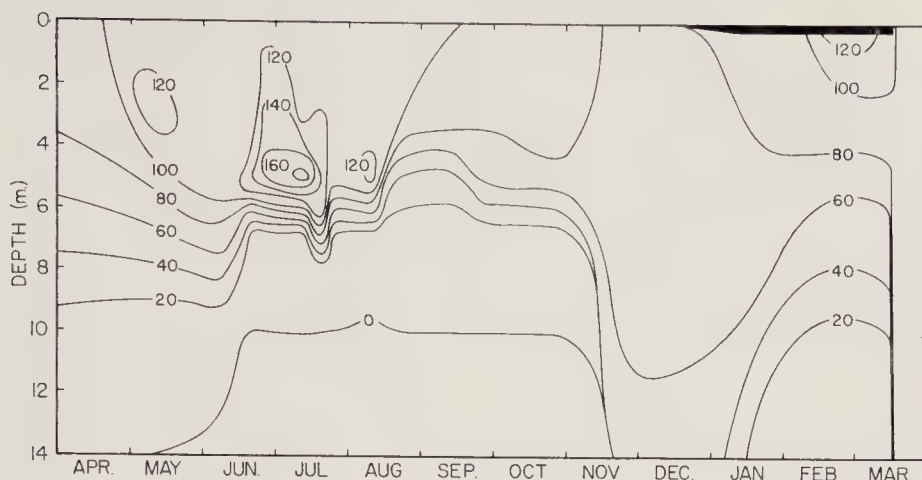


FIG. 19. Isopleths of percentage saturation of dissolved oxygen, Station A, Goose Lake, 1963-1964. Opaqued area = ice cover.

mg/l (131.5% saturation) of oxygen were reached at 3 m in the then relatively shallow metalimnion of 1.5-3.5 m (Fig. 17-19). Following this peak in productivity, oxygen levels within the metalimnion were reduced but still remained near 120% saturation at the existing temperatures. A very intensely developed oxygen maximum began in mid-June, persisted until late July, and again developed weakly for a brief period in August. A maximum oxygen concentration of 16.6 mg/l and 189.5% saturation was found at 5 m on 11 July. As discussed in the above section, the primary productivity of the metalimnetic region was particularly intense throughout the periods of the maxima in direct correlation with the development of high oxygen concentrations.

Considerable depletion of alkalinity occurred in the epilimnion and especially the metalimnion during periods of maximal metalimnetic productivity (Fig. 20). While such removal from the surface waters is largely biogenic in origin, the depletion beneath the terminal phases of ice cover is more likely a result of dilution, as discussed above, than of biological utilization and precipitation of carbon sources. As shown above,

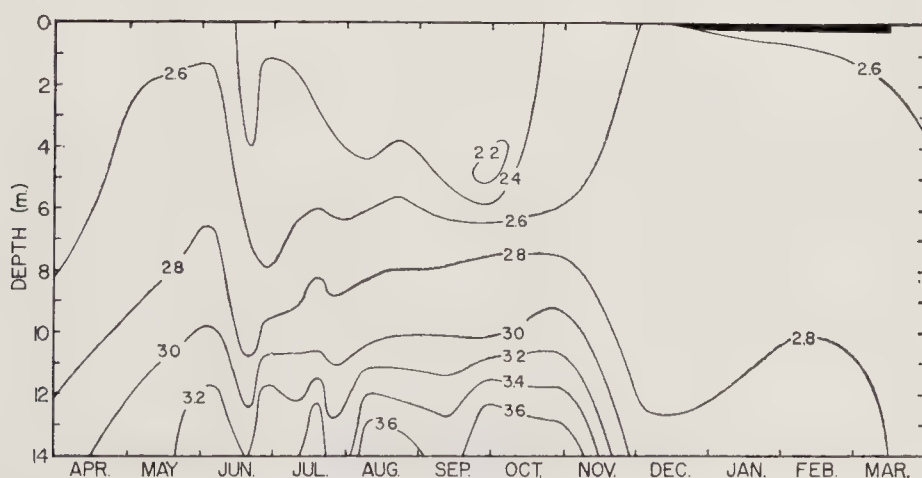


FIG. 20. Isopleths of alkalinity in milliequivalents per liter, Station A, Goose Lake, 1963-1964. Opaqued area = ice cover.

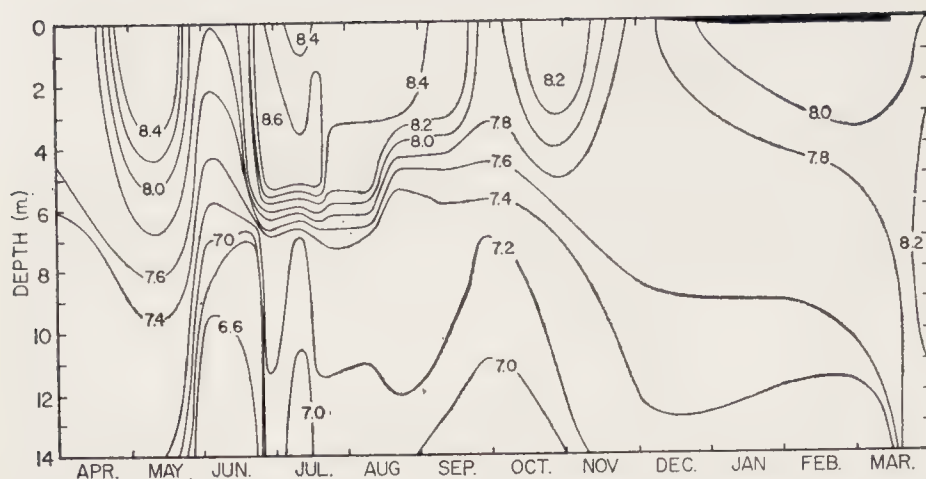


FIG. 21. Isopleths of pH, Station A, Goose Lake, 1963-1964. Opaqued area = ice cover.

however, a large population and productivity developed at this time, which was reflected in the oxygen concentrations and levels of pH.

The major ionic concentrations of Goose Lake are typical for many of the hard-water lakes of northeastern Indiana. Very high calcium concentrations and approximately equivalent amounts of sodium and potassium prevailed among the cations with a general order of $\text{Ca} > \text{Mg} > \text{Na} > \text{K}$ (Table 8). The relative proportion of anions deviated somewhat from the usual for hard-water lakes in that sulphate was somewhat greater than chloride in the order $\text{CO}_3 > \text{SO}_4 > \text{Cl}$. Deviation was not great, however. Concentrations of minor elements of the surface water in mid-summer (Table 9) were well within the levels commonly found in lakes of the temperate zone (Livingstone, 1963; Hutchinson, 1957). Concentration of molybdenum was quite high in comparison with the few analyses available by other workers.¹ Analyses of concentrations of minor elements from surface waters of several other lakes in northern Indiana (Wetzel, 1966) demonstrated considerably higher values for molybdenum, however, than those reported here for Goose Lake.

DISCUSSION

Sylvan Lake

The autotrophic productivity of Sylvan Lake is almost entirely planktonic in origin. A few sparse macrophytes (*Potamogeton*) occur in the immediate inlet area, but their distribution is rapidly limited by plankton-induced light attenuation at a depth of approximately 10 cm. Emergent vegetation, primarily *Scirpus*, occurs sparsely in the relatively few undisturbed areas and mainly in Area B near the mouth of this arm of the lake.

Inhibition of photosynthesis near the surface has been observed by many workers during *in situ* determinations of primary productivity.

¹ The mean of 0.84 ppm cited by Livingstone (1963) is likely a typographical error for ppb.

TABLE 8. Ionic analyses of water from Goose Lake, Station A, Kosciusko County, Indiana. Values are given in mg per liter.

Date	Depth (m)	Ca ⁺⁺	Mg ⁺⁺	K ⁺	Na ⁺	Cl ⁻	SO ₄ ⁼	Total PO ₄ ⁼	NO ₃ ⁻	Mn ⁺⁺	Fe ⁺⁺⁺
2 Aug. 1963	7	44	10	3	5	4	13	—	—	—	—
27 Oct. 1963	1	39	18	3	2	6	15	<0.05	—	—	—
	13	49	16	3	2	6	15	2.3	—	—	—
31 Jan. 1964	7	42	11	3	3	5	13	0.3	—	—	—
29 Feb. 1964	1	42	9	3	4	5	14	—	0.2	<0.05	0.05
	13	44	11	3	3	6	10	—	<0.05	1.2	0.04
26 Mar. 1964	1	42	9	3	4	3	12	0.1	0.2	0.1	0.02

TABLE 9. Concentration ($\mu\text{g/l}$) of minor elements at 1 m, Station A, Goose Lake, Kosciusko County, 3 July 1964.

Ag	<0.3	Be	<0.03	Fe	17.	Pb	8.
Al	8.	Cd	<5.	Mn	20.	Sr	31.
As	<40.	Cr	<1.	Mo	30.	V	<5.
B	19.	Co	<3.	Ni	<3.	Zn	6.
Ba	16.	Cu	<2.	P	15.		

Such inhibition is undoubtedly influenced by high light intensities. Considerable evidence is accumulating that much of the reduction in photosynthesis is a result of photo-oxidation of chloroplast enzymes rather than an actual destruction of chloroplasts (discussed at length by Wetzel, 1964a). Surface inhibition of photosynthesis was observed very rarely in Sylvan Lake and then only of minor magnitude (*cf.* Fig. 3). Vertical extinction of light is effectively accomplished by dense plankton populations and is directly density dependent (Pearsall and Ullyott, 1934, and especially Talling, 1960). The rapid attenuation of light intensity, and consequent reduction of photo-inhibition, by high biogenic turbidity is probably a very important factor in the high levels of photosynthesis achieved in the first few centimeters of the water in a hypereutrophic situation (*cf.* Wetzel, 1966).

Extremely high values of primary productivity of Sylvan Lake persist throughout the year. Although reduced under ice cover and considerably more stable during this period, the mean daily winter values are still far above those annual daily means and often summer daily means of a majority of natural waters of this latitude. The contribution of the winter period to the total annual productivity, and consequently to the annual mean daily values, is of significance, and this portion cannot be neglected as has frequently been the case (*cf.* Vinberg, 1960). The magnitude of winter productivity is accentuated in highly productive waters such as Sylvan Lake by the persistent light-induced restriction of planktonic growth to the immediate surface layers. In the undisturbed stratification beneath ice cover, productivity continues at relatively high rates, in spite of reduced temperatures, in a distributional pattern similar to that occurring during most of the year.

The greater rates of growth per cubic meter and more dense populations of the more productive areas of the lake rapidly limit the depth of the trophogenic zone to very shallow compensation depths. These effects of high variation in spatial distribution and volumetric rates of productivity compensate for the differences, however, and result in a relatively uniform productivity on an areal basis. Therefore, the variations in population pulses, densities, and rates of growth in different parts of the lake average out to relatively consistent accumulative productivities on an integrated annual basis. The extreme productivity in certain areas, however, points out an obvious example of the impact of excessive growth as a self-limiting metabolic mechanism.

The efficiency of photosynthetic utilization of visible radiation further emphasizes the limiting effect of excessive growth. The photosynthetic efficiency was calculated assuming that 50% of the photo-

synthetic production is carbon and has a heat of combustion of 5.5 kcal/g (*cf.* Ryther *et al.*, 1958) and further that half of the incident radiation is photosynthetically effective (Strickland, 1958). The photosynthetic efficiency was greatest in Area A, with an annual mean of 1.13% (range of 0.04-2.41%), where algal densities were less than in the other areas. Efficiencies of light utilization were reduced considerably in areas B and C, with annual means of 0.90% (range, 0.004-2.27%) and 0.91% (range, 0.20-2.74%), respectively. While the shading effects of bloom conditions, especially among surface-dominant bluegreen algae, have a beneficial effect on the population in the rapid reduction of photoinhibition, the efficiency of energy utilization can be reduced if excessive densities of plankton develop.

Surface aggregations of bluegreen algae, mainly *Anabaena*, in littoral areas and bays of Sylvan Lake frequently become a nuisance. A local cottage-owners association applied approximately 1350 kg of copper sulphate (as a solution of *c.* 7.7 g Cu/l) during the summer of 1963 to near-shoreline and small localized areas. Although evidence is meager (Hutchinson, 1957), considerable loss of ionic copper occurs by means of the formation of copper carbonate in hard waters such as Sylvan Lake. Fogg and Westlake (1955) demonstrated convincingly that it is highly probable that extracellular polypeptides of some algae, especially certain myxophyceans, chelate cupric ions to form non-toxic complexes in nature and hence inactivate much of the copper applied to lake water. It is likely that the applications of copper had no significant effect on the algal populations of Sylvan Lake as a whole and were of limited value except in very localized situations.

The striking correlation between the sustained high levels of photosynthesis by the bluegreen algae and abnormally high concentrations of sodium give added credence to the beneficial effects of Na to the growth of these forms. The Na requirements are known to be high and very specific for the bluegreens (Allen, 1952; Gerloff *et al.*, 1952; Allen and Arnon, 1955). Potassium and other metals of this series (Li, Rb, and Cs) cannot substitute for Na. A threshold level of 4 mg Na/l is required for near optimal growth (Kratz and Myers, 1955), a concentration that is about the mean for a majority of the lakes of this region and likely controls, among other factors, the development of large populations of this algal group (Wetzel, 1966; Wetzel and Lipscomb, 1967). Maximal growth of several bluegreen forms was found at levels of 40 mg Na/l (Kratz and Myers, 1955), well below that of Sylvan Lake (Table 6), and there is no evidence that higher concentrations are harmful. These data, in combination with the high levels of phosphate, indicate poignantly that Provasoli's (1958) suggestion of Na as an important contributor to blooms of bluegreen algae in enriched waters is true. The combination of organic materials, and especially phosphorus and sodium, from the increased use of detergents from urban sources favors the prolific development of these algae and lake enrichment. To truly prevent or reduce rapid lake eutrophication it may well be necessary to remove in sewage treatment more than the various organic compounds traditionally considered to be responsible for blooms of bluegreen algae.

Goose Lake

The extent of the development of metalimnetic oxygen maxima and positive heterograde curves was not nearly so great in Goose Lake as has been recorded by many other workers for other waters (*cf.* reviews by Hutchinson, 1957, and especially Eberly, 1964). The origin of the two distinct maxima in this lake is certainly biogenic, as confirmed by the assays of phytoplankton productivity. Development of such maxima is obviously the result of the interaction of a multiplicity of environmental factors, including morphometry, degree of basin protection, meteorological conditions, transparency, and distribution of nutrient concentrations, to name but a few. The relative depth (Z_r) of Goose Lake of 3.55% and small surface area are in accord with Eberly's (1964) attempt to correlate morphometric characteristics with metalimnetic oxygen maxima. Conditions of temperature, transparency, and underwater light intensity are also in good agreement with those lakes in which many maxima have been found. Such correlations are difficult, however, and plagued with many weakening exceptions dependent on local variations in edaphic conditions. The development of oxygen maxima is undoubtedly variable from year to year in lakes of marginal characteristics, as Goose Lake appears to be in this respect. Conceivably in Goose Lake such maxima may develop to a greater extent than found in the present study or be below the pre-stratification saturation value in other years. There is considerable evidence (Rodhe, 1958; Wetzel, 1966) that primary productivity is relatively constant from year to year on an integrated annual mean basis, provided the lake is not radically altered by the activities of man. Because of the resultant nature of such annual mean values that integrate a host of interrelated parameters governing growth, they provide one of the best measures of the degree of lake trophy yet available. To be emphasized again, however, is the utmost necessity of determining such values on an annual basis; the winter period cannot be neglected. In correlations of productivity, as well as other characteristics of the lake, to the occurrence and development of metalimnetic oxygen maxima, especially permanent climax types, annual mean daily values would be preferable to irregular isolated daily determinations.

It is difficult to place the type of oxygen maxima of Goose Lake into the categories suggested by Eberly (1963, 1964). In some of the characteristics of the oxygen regime, the maxima would be classified as "temporary sub-climax" in that it did not persist until autumn circulation. Although it is likely in many cases that the disappearance of such a maximum in mid-summer is due to depletion of nutrients in the metalimnion, there is no evidence for this either in the case of Goose Lake or in the work of Eberly. Other factors, such as the qualitative shifting of phytoplankton populations, may also be involved, particularly in intermediate stages of the development of oxygen maxima.

Some characteristics of the oxygen maxima of Goose Lake would place the lake in the "*Oscillatoria* maximum" subclass of the "permanent climax" oxygen maximum type. A stenothermal *Oscillatoria* community occurred, but this alga was not the dominant form. The zone of oxygen maximum was constricted, and percentage levels of surface light in the metalimnion fell into the latter subclass. Transparency was relatively

low. The moderate metalimnetic oxygen maximum of Little Crooked Lake, a short distance to the northeast of Goose Lake and somewhat similar in morphometric characteristics, persisted throughout the latter portion of stratification (Wetzel, 1966). In Little Crooked Lake the maximum was much more pronounced in 1964 than in the previous year, when it was barely evident, which again points out the variability that can occur in lakes that are marginal in development of oxygen maxima. It is very probable that the development of metalimnetic oxygen maxima within a broad spectrum of intensities and stages is not the exception in many small, protected lakes of continental glaciated regions but rather that this phenomenon is quite common. As useful as many systems of classification are, constant caution is essential in strict application of artificial systems to ecosystems of individual entity and a broad spectrum of transitional stages.

The partial and temporary meromictic circulatory pattern of Goose Lake is the result of dynamic processes (Findenegg, 1937) of decomposition and sedimentary solubilization in the lower levels of the lake under a combination of morphometric and unusual meteorological conditions. Such a temporary biogenic meromixis, using the definitions of Hutchinson (1937, 1957), in Goose Lake during the period of investigation was probably initiated during the lengthy winter of 1962-1963, during which deep water of the monimolimnion accumulated relatively large quantities of dissolved inorganic matter thereby increasing its density. Warming of the superficial layers was rapid and probably created density gradients too great to permit complete circulation into the chemically enriched lower strata. Accumulation continued throughout the summer and autumn periods of relatively high productivity, further increasing the levels to a point where the existing meteorological conditions were not sufficient to overcome the gradient completely prior to rapid ice formation in mid-December. In his classical work on phosphorus cycles and enrichment effects in the small, eutrophic lake Schleinsee, Einsele (1941) found that complete spring turnover occurred only every other year on the average. As Hutchinson (1957) points out, such a suppression of vernal circulation is probably common in small, deep, sheltered lakes in areas of severe winters. Circulation in Goose Lake was complete in the spring of 1964, however, during a period of severe wind action.

Partial, temporary meromixis as described above has been similarly found in two other lakes of the 13 in this investigational series (Wetzel, 1966). While differing in several respects from that of Goose Lake and in one case more pronounced, the morphometric characteristics are analogous. It is likely that lakes possessing an intermittent biogenic meromixis are very common among the glaciated terrain of the continental midwestern United States with its severe winters.

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Rate of Digestion in the Bluegill Sunfish¹

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ABSTRACT

This study is an initial attempt to compare the rate of digestion and absorption of several different natural food organisms by the bluegill sunfish (*Lepomis macrochirus*). The rate of digestion was measured as the difference between the weight of food intake and food remains in terms of digestible organic matter. Similar results were obtained when total protein digestion was measured by means of micro-Kjeldahl nitrogen determinations.

Meals of natural food organisms required about 18 hours for complete digestion by the bluegill. Digestion was most rapid during the first six hours, with the digestible organic matter in the stomach decreasing by about 50%. Meals of mayfly naiads, crayfish, and darters all reached 100% digestion after 18-hours, whereas chironomids and oligochaetes reached 92.9 and 97.8%, respectively. A meal of three dragonfly naiads, because of a drop in temperature, reached only 85% digestion after 18 hours.

The rate of digestion of a voluntarily consumed mixed meal of 25 chironomids, 1 dragonfly naiad, and 1 oligochaete showed that only 15% of the food remained in the stomach after 10 hours. No differences were observed in the rate of passage or relative digestibility of the different food organisms. Chitin lingered in the stomach until being broken into small pieces before passing through the pyloric valve.

Special studies were conducted on the effects of force-feeding, starvation, body size, and size of the meal on the rate of digestion. Bluegills that were force-fed mealworms showed considerable individual variation in digestion when compared with control fish that voluntarily consumed meals. The variation was great enough so that the outcome of subsequent experiments could not be predicted accurately. Bluegills starved for 7, 14, and 25 days showed a progressive decrease in rate of digestion when compared with control fish. The decreased digestive efficiency appeared to be a result of shrinkage and other morphological changes that occurred in the pyloric caeca. A comparison of the combined average differences in percentage digestion at each of the sacrifice intervals showed that control fish digested 1.25, 1.85, and 2.22 times faster than fish starved for 7, 14, and 25 days, respectively. Comparison of the rate of digestion of single- and multiple-unit meals by fish of the same relative age and weight showed that the stomach empties at about the same rate. As the size of the meal was increased by 2.7 times, the rate of digestion per unit time increased 2.2 times. Among fish of different ages and body weights, the time required for the stomach to empty of single- and multiple-unit meals was not significantly different.

A method is presented for establishing the "maximum daily ration" for fish in the laboratory. Comparisons of laboratory and field information will lead to ecological predictions concerning both fish and fish-food populations in relation to growth and production in aquatic ecosystems.

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TABLE OF CONTENTS

	Page
Introduction	186
Methods	188
Collection of fish	188
Food organisms	188
Conditioned feeding	189
Definition of meals	189
Size of the meals	189
Method of autopsy	189
Organic weight analysis	190
Chitin analysis	190
Total nitrogen analysis	190
Temperature	190
Results	191
Chemical analysis of food organisms	191
Digestion of mealworms	193
Digestion of single- and multiple-unit meals	193
Digestion of proteins	194
Force-feeding	195
Age and size of the fish	198
Starvation	199
Digestion of natural food organisms	202
Digestion of a voluntarily consumed mixed meal of natural food organisms	204
Absorption	206
Discussion	207
Factors influencing rate of digestion	207
Handling and fish sensitivity	207
Digestion of large and small meals	208
Meal succession	208
Differential rates of digestion of natural food organisms	209
Starvation and the winter period	209
The daily ration	210
Acknowledgments	212
References	212

INTRODUCTION

The present work is a study of gastric digestion of the bluegill sunfish (*Lepomis macrochirus*). The amount of food digested per unit time will be useful in estimating the daily ration and in estimating the time required for the disappearance of food present in stomachs in nature. This problem was recognized by Ricker (1946) as an important area of study bearing on fish production. Since that time, only a very few studies have appeared on the subject. The major emphasis in the present work is to learn the rate of digestion of organisms that might be encountered in the environment.

The research on the general subject of gastric digestion in fishes can be broken down into two categories: (1) food habits and (2) digestive enzymes. The rate of digestion of natural food organisms will bridge a gap between the two. Specific work on the digestion of food by fish dates back to the work of Spallanzani and Senebrier (1783). Digestion was studied by utilizing tubes filled with flesh, placing these into the stomachs of various species, and recovering them at intervals. Essentially the same technique was used by Riddle (1909), who used small glass tubes (Metts Tubes) filled with coagulated egg protein. Various other

approaches have been used since these early studies. Van Slyke and White (1911) performed the only attempt to measure biochemically the rates of digestion and absorption of protein after force-feeding chopped and boiled beef. Markus (1932), after force-feeding minnows, injected a nitrous acid solution at various time intervals, thereby inducing regurgitation of the remaining stomach contents. Several workers have attempted to measure digestion by placing a variety of substances, including natural food organisms, into prepared extracts of digestive juices, acids, and alkalis (Battle, 1935; Bayliss, 1935; Jancarick, 1964). A larger number of workers have reported rates of digestion for various species using arbitrarily defined stages of digestive disintegration (Pierce, 1936; Sokolov and Chvoliova, 1936; Karpevitch and Bokoff, 1937; Hess and Rainwater, 1939; Webster, 1942; Plantenburg, 1961; Darnell and Meierotto, 1962). An X-ray method was designed to follow the process of gastric digestion from which rates were calculated by Molnár and Tölg (1960, 1962). The volumes of the meals and the partially digested food recovered from the stomachs have been measured by volume of water displacement (Hunt, 1960; Seaburg and Moyle, 1964). Because of the variety of methods used, there are few data available for direct comparison between species. The present study is an initial attempt to compare the rate of digestion of several different natural food organisms. This was done with the hope that results obtained under field conditions could be more readily interpreted.

The rate of gastric digestion is defined here as the rate at which the digestible organic matter disappeared from the stomach. Digestion is considered to be complete when the stomach becomes empty of all measurable remains. In this study the rate of digestion was measured for the first time as the difference between the weight of food intake and the remains in terms of digestible organic matter. By feeding a known ration, the percentage decrease of digestible organic matter from the stomach was periodically measured after sacrifice and autopsy of a number of animals. Additional measurement of dried intestinal remains provided values for the estimation of gastric and intestinal absorption.

In these experiments, bluegill sunfish digested meals of natural food organisms in about 18 hours at 21°C. Digestion was most rapid during the first six hours, during which time the digestible organic matter decreased by about 50 percent. Similar results were obtained when total protein digestion was measured by means of micro-Kjeldahl nitrogen determinations. Crayfish, oligochaetes, chironomids, mayfly naiads, and darters were all digested at about the same rate even though some are considered "hard-bodied" and some "soft-bodied" organisms. The amount of chitin in the exoskeleton evidently plays only a minor role in inhibiting the rate of digestion.

While the determination of the differential rates of digestion of natural food organisms was the main purpose of this study, certain important factors that affect the rate of gastric digestion of the bluegill required investigation. Special studies were conducted on the effect of force-feeding, starvation, body size, and size of the meal.

Compared with control fish that voluntarily consumed meals, bluegills that were force-fed mealworms showed considerable individual varia-

tion in digestion. In most cases individual variation was great enough that the outcome of future experiments could not be predicted accurately. One of the most striking results of the study was the relationship of starvation to digestion. Fish that were starved for 7, 14, and 25 days showed a progressive decrease in the rate of gastric digestion when compared with control fish. Comparison of the rate of digestion of single- and multiple-unit meals by fish of the same relative age and weight showed that the stomach empties at about the same rate. Neither was there any difference in the rate of digestion of single- and multiple-unit meals by fish of different ages and different body weights.

METHODS

The rate of digestion was studied using several variations of the same general method. The specific details concerning the organization of the different experiments, which varied according to the particular problem to be solved, are given before the results are described.

Collection of fish. Most of the bluegill sunfish used in the following digestion experiments were captured by seining from Winona Lake in Kosciusko County or Sylvan Lake in Noble County, Indiana. The bluegills used to test the effect of body size on the rate of digestion were obtained by trapping from Shriner Lake in Whitley County.

The fish were transported in aerated water tanks to the Indiana University Biological Station on Crooked Lake, Noble County, where they were temporarily held in large tanks containing aerated, unfiltered lake water. After this initial holding period, the fish were selected on the basis of size and physical appearance and placed individually in 15-gallon aquaria.

The starvation and force-feeding experiments were carried out in the aquarium room of the Indiana University Zoology Department, Bloomington, during the autumns of 1963 and 1964. The bluegills in these experiments were caught by seining at the Driftwood Experimental Station in Washington County and at the Avoca Fish Hatchery in Lawrence County. They were handled and cared for as described above, with the exception that charcoal-filtered tap water was used in the aquaria.

The experiments were started by 15 June in 1963 and 1964, and no experiments were carried out after 15 October of either year to eliminate any possible difficulty with temperature acclimation. The average number of days that the fish were held in the laboratory from collection to sacrifice, excluding the starvation experiments, was 11 days.

Food organisms. The larvae of the darkling beetle (*Tenebrio molitor*) were cultured in large tanks on commercial chick mash and used as food in many of the experiments. In addition to mealworms, six natural food organisms were used as food. These organisms included aquatic insect larvae belonging to the families, Tendipedidae (*Chironomus* sp.), Ephemeridae (*Hexagenia* sp.), and Libellulidae (*Libellula* sp.), one species of crayfish (*Cambarus* sp.), a darter (*Etheosteoma* sp.), and an oligochaete (*Lumbricus* sp.). Each was collected from its natural habitat, brought to the laboratory, and held in aerated aquaria.

Conditioned feeding. Digestion experiments have encountered difficulty in the past because of the failure to train fish to feed voluntarily. For this reason, most workers have resorted to force-feeding. Some investigators (Hathaway, 1927; Sokolov and Chvoliova, 1936; Ricker, 1949; Gerking, 1952, 1955; Baldwin, 1957), however, have been successful in training game fish to feed voluntarily in laboratory aquaria. With this in mind, it was essential that a method of conditioned feeding be explored before attempting the experiments.

The bluegill sunfish quickly establish territories in large holding tanks. A few individuals become aggressive and dominant, while most remain subordinate. The dominant individuals nip the tails of the subordinates, and the latter are badly injured in a relatively short time. When feeding is attempted, the dominant fish immediately consumes as much food as possible and will guard any that remains.

With the above observations in mind the following procedure was carried out with excellent success. Fish were kept for one day in large holding tanks before adding small amounts of food. The aggressive fish generally ate after a few attempts at feeding, but if the food was not eaten by the dominants in a short period of time it was removed. It is important that the subordinates observe the dominants feeding. The fish were then placed individually in separate aquaria, which were arranged side by side and end to end so that the subordinates could again observe the dominants feeding. Food was offered to all fish at intervals and removed if it was not consumed within a short period. Consequently, the many subordinate fish were "teased" into feeding on the desired food organism for each experiment. This procedure made it possible to train large numbers of fish to consume meals simultaneously. Considerable caution was taken not to startle the fish during the feeding period.

An attempt to condition bluegills to feed on small live darters by this method proved to be unsuccessful. This failure may be explained partially by the fact that the age-group used in these experiments is not normally piscivorous in nature.

Definition of meals. Both voluntary and force-feeding experiments employed either a single-unit meal consisting of one food organism or a multiple-unit meal which included more than one food organism.

Size of the meals. The fish were fed on the basis of their beginning body weight. Gerking (1955) fed daily rations of mealworms to bluegills at rates varying from 1 to 3.5% of the body weight per day over extended periods. Observation and preliminary analysis showed that meals equaling about 1.5% of the body weight could be consumed at one feeding.

Method of autopsy. Once all fish were thoroughly conditioned to consume an experimental meal voluntarily, a three-day fasting period was initiated. This insured the complete absence of food remains from the alimentary canal. After this fasting period the fish were individually fed either a single-unit or multiple-unit meal weighed to the nearest 0.1 mg.

The experimental fish were then sacrificed at time intervals of 6, 10, 14, 18, and 22 hours after feeding. The esophagus, pylorus, and anus were ligated with hemostats to prevent loss of contents. The organs were removed from the abdominal cavity and the food remains removed

from the stomach and intestine and placed in separate crucibles. The crucibles containing the remains were dried in a drying oven until a constant dry weight was achieved and then removed to a desiccator. They were weighed on a Mettler balance sensitive to 0.01 mg.

No difficulty was encountered in the removal of stomach contents. The esophagus was cut immediately posterior to the hemostat. The contents of the stomach were squeezed into a crucible. This technique generally removed all stomach remains; however, in each case the stomach was cut open, inspected, and any remaining food was removed by means of forceps.

The removal of the intestinal remains involved the use of a stripping action. Using the thumb and forefinger, the entire intestinal tract can be stripped completely free of all contents. The transparency of the intestine aids in assuring complete removal.

Organic weight analysis. Each food type was analyzed to determine the relative amounts of digestible organic matter, chitin, and total nitrogen. The live weight was determined for each food type, and a quantity of the organism was placed in a drying oven at a temperature of 105°C until a constant dry weight was achieved. The dried material was then heated in a muffle furnace at a temperature of 600°C for a period of 24 hours, and the remaining white ash was weighed. The weight of organic matter was obtained by subtracting the ash weight from the dry weight, and the corresponding percentage was calculated.

Chitin analysis. The amount of chitin relative to the live weight was determined for each chitinized food organism and subtracted in calculations of the rate of digestion. The procedure given by Richards (1951) for the quantitative estimation of chitin was followed. This method involves purification with 5-20% NaOH at 50-100°C for some hours, followed by washing in water and a dilute acid. The material is then treated with an oxidizing agent followed by a solution of Na₂SO₃, which oxidizes the more alkali resistant components. A succession of thorough washing with water, alcohol, and ether was followed by complete drying.

Total nitrogen analysis. Total nitrogen was used as a measure to calculate the rate of protein digestion in the stomach. The analyses were performed on finely ground material dried to a constant weight. Duplicate samples were run, and appropriate blank and standard values were obtained. The digestion mixture recommended by Bruël *et al.* (1947) was used in a rotary micro-Kjeldahl digestion apparatus. Nitrogen determinations were made by following the procedure of Niederl and Niederl (1942), using a steam distillation micro-Kjeldahl apparatus of both the Pregl-Parnas-Wagner and American Instrument Company designs. The distillate was titrated with an automatic filling microburet. Acetanilide, secured from the U. S. Department of Commerce, National Bureau of Standards, was used as the micro-chemical standard (10.36% nitrogen).

Temperature. Food consumption and digestion rates in fishes are known to vary with respect to many factors including temperature (Markus, 1932; Sokolov and Chvoliova, 1936; Baldwin, 1957; Malcolm, 1960; Molnár and Tölg, 1962). The temperature was recorded throughout the entire holding period for each experiment using maximum-minimum

and standard centigrade thermometers. The water temperature range for the mealworm experiments from the time the experimental meal was fed to the time of sacrifice was 19.0-24.1°C (mean = 21.6°C). The water temperature range during the natural food experiments was 18.5-24.6°C (mean = 21.3°C).

RESULTS

Chemical analysis of food organisms

The problem of obtaining adequate supplies of live organisms has been a factor prohibiting large scale digestion experiments. Many workers have been forced to use foods such as chopped and boiled beef, coagulated egg white, ham, lettuce, and various mixtures. These foods are easy to obtain and handle, but may bear no relation to the organic makeup of organisms encountered in nature by the bluegill. Therefore, it is not unreasonable to emphasize the importance of using natural foods consumed voluntarily to determine accurate rates of digestion. The organisms used in the present study included chironomids, crayfish, dragonfly naiads, mayfly naiads, darters, and oligochaetes.

The chemical composition of the food organisms consumed is an important variable in experiments on fish digestion. The physical-chemical composition of the food organisms probably plays an important role in the rate of digestion of fishes. The relative amounts of fats, proteins, carbohydrates, chitin, scales, and bone cannot be ignored if valid values of digestion rates are to be determined.

The seven different food organisms used in the present experiments was each analyzed for a number of chemical components (Table 1). The live weight of the food organisms was determined after a period of starvation, which eliminated all contents from their alimentary tracts. This can amount to significant error if not considered for such organisms as chironomids and oligochaetes. Moreover, the amount of water present in various body cavities can cause serious error in establishing accurate dry weight and live weight percentages. Considerable difficulty was encountered in removing water from the rectal gill of the dragonfly

TABLE. 1. Chemical composition of food organisms used in digestion experiments.

	Live weight per individual (mg)	Water %	Organic matter related to live wt %	Chitin related to live wt %	Nitrogen related to dry wt %
Chironomid	24.5	87.2	10.5	0.43	10.3
Crayfish	94.9	79.8	15.3	1.07	—
Dragonfly naiad	84.6	80.4	18.2	2.45	11.7
Mayfly naiad	207.1	80.9	17.9	1.42	—
Mealworm	141.4	59.7	38.0	1.76	9.3
Darter	89.5	77.5	18.9	—	—
Oligochaete	233.9	84.4	14.8	—	11.5

naiad and the gills of the crayfish. Each individual was thoroughly blotted before weighing. The average individual body weights ranged from the smallest of 24.5 mg per chironomid to the largest of 233.9 mg per oligochaete.

The dry weights exhibit a relatively close correspondence with the exception of the laboratory-grown mealworm. Their low water content of 59.7% is probably a result of being cultured on a dry commercial chick mash. Chironomids have a slightly above average water content of 87.2%.

The percentage water reflects much of the remaining body composition. As would be expected, mealworms have an extremely high organic weight of 38.0%. This value is over twice as high as that recorded for darters, which have the next highest organic weight of 18.9%. The high water content of chironomids causes a low organic weight of 10.5%. Only small differences were found in the organic weight percentages of the remaining food organisms.

The proportion of chitin was determined for the chitinized organisms. These values were used to calculate the amount of chitin in each meal. The chitin value for chironomids of 0.43% was slightly lower than the average, whereas the dragonfly naiads have a significantly higher chitin content of 2.45% of the live weight. The importance of determining chitin is obvious, since chitin is generally considered to be indigestible by fish and, therefore, is egested quantitatively (Geng, 1925; Battle, 1935; Mann, 1948; Gerking, 1952). Hess and Rainwater (1939) reported finding sclerites from the exoskeleton of heavily chitinized stonefly nymphs in trout stomachs 95 hours after force-feeding, suggesting the possibility that continued feeding might cause these indigestible parts to move through the alimentary tract more rapidly. Gerking (1952) observed that a large proportion of the fish feces consisted of small pieces of integument from the mealworm larvae that were fed in his experiments. Mann (1948), feeding a mixture of cladocera and copepods to carp, reported similar observations. This was also found to be true in the present experiments using chironomids, crayfish, dragonfly naiads, mayfly naiads, and mealworm larvae. Over a period of time, considerable quantities of small chitin chips accumulate on the bottom of the aquaria.

The proportion of nitrogen enables the amount of protein consumed to be calculated. Relatively small differences exist among the various organisms analyzed. Dragonfly naiads had the highest nitrogen content of 11.7% dry weight with oligochaetes, chironomids, and mealworms having values of 11.5, 10.3, and 9.3%, respectively (Table 1).

The importance of fish scales in affecting digestive rates was not determined, but appeared to be an insignificant factor in the darter experiment. It was observed from preliminary experiments and observations that scales are not digested by the bluegills but pass through the alimentary tract unchanged. Markus (1932) reported similar observations for the largemouth bass (*Micropterus salmoides*), which expel the scales through the mouth and gill openings. Battle (1935) found the stomachs of herring gorged with fish scales. A basic extract from the pyloric caeca had no effect on the scales even over a period of months.

Digestion of mealworms

Digestion of single- and multiple-unit meals. Reliable rate-of-digestion comparisons can be made only when the amount of food consumed is controlled. Several workers emphasize the importance of meal size on digestion rates, but the evidence is often based on subjective judgments (Pierce, 1936; Hess and Rainwater, 1936). Hunt (1960) showed quantitatively that Florida gar (*Lepisosteus platyrhincus*) were able to digest a single *Gambusia* weighing 0.5 g in 25 hours, whereas 42 hours were required to digest five force-fed *Gambusia* weighing 2.5 g. The following experiment was designed to determine the rate of digestion for bluegill sunfish utilizing meals of two different quantities of mealworm larvae.

A collection of 50 fish was divided into two groups. One group was allowed to consume voluntarily a single mealworm, whereas the second was offered a multiple-unit meal of three mealworms each. Both groups were treated and handled in a similar manner under identical laboratory conditions. The amount of digestible organic matter consumed per fish (Table 2) with each meal was calculated by using the value of 38% organic matter previously determined by chemical analysis (Table 1). It varied from 71 mg in the single-unit meal to 193 mg in the multiple-unit meal.

Although different amounts of digestible organic matter were fed with the single- and multiple-unit meals, only small differences occurred in the rate at which the stomachs emptied (Fig. 1). The largest difference occurred at 6 hours; the stomach contents of the fish that consumed a single-unit meal decreased by 31%, while those fed the multiple-unit meal decreased by only 19.5%. The percentage decrease of stomach contents for the remaining fish that consumed a multiple-unit meal was 35.4, 52.5, 69.5, and 81.8% for the 10-, 14-, 18-, and 22-hour intervals, respectively. The average percentage decrease for those fish consuming a single mealworm was 42.2, 55.1, 70.0, and 85.5% for the corresponding sacrifice intervals.

Single- and multiple-unit meals of mealworms require more than 24 hours for complete digestion by the bluegill. The close relationship of the two curves implies that food disappears from the stomach at approximately the same rate regardless of the amount of digestible organic matter consumed.

TABLE 2. Experimental data for bluegills fed a single-unit and multiple-unit meal of mealworms.

	No. mealworms	Av. fish length (mm)	Av. fish weight (g)	Av. live weight of food (mg)	Av. organic weight of food (mg)	Food as % of body weight	
						Live weight	Organic weight
Single-unit meal	1	126.7	36.8	187.0	71.0	0.508	0.193
Multiple-unit meal	3	125.8	34.8	507.5	192.5	1.46	0.553

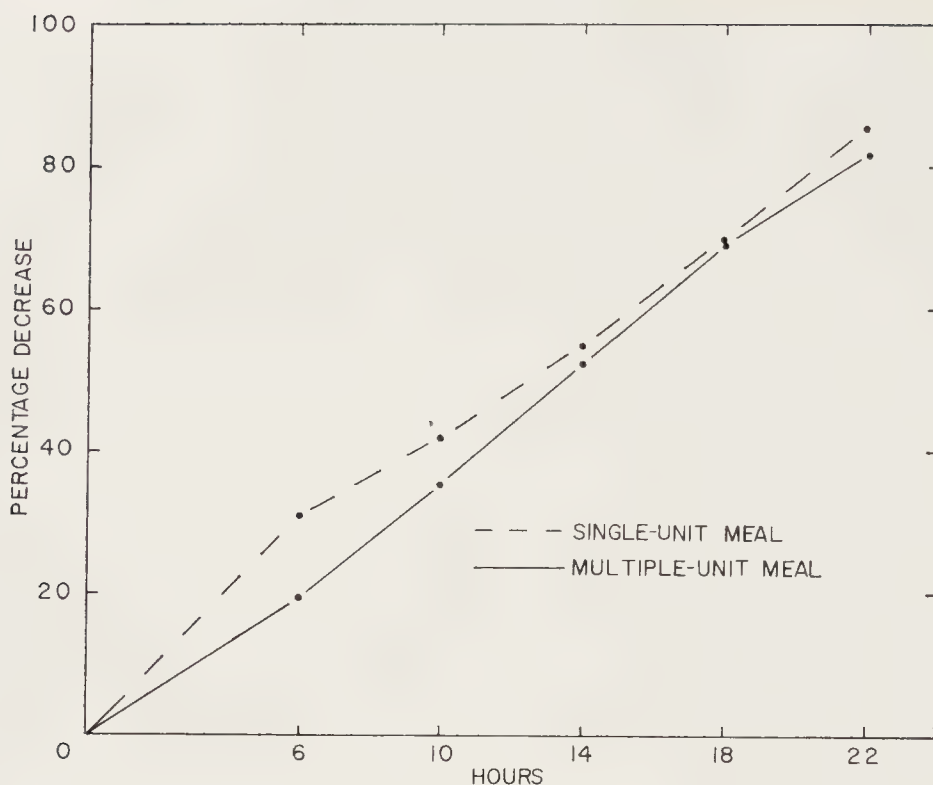


FIG. 1. The average percentage decrease of digestible organic matter from the stomach of the bluegill sunfish fed single- and multiple-unit meals of mealworms. Each point represents an average of five fish.

The room temperatures, from the time the experimental meal was fed to the time of the last sacrifice, averaged 20.8°C for fish consuming the single-unit meal and 23.1°C for the bluegills consuming the multiple-unit meal. Although these differences are small, the values for the multiple-unit meal may be slightly high in relation to the results for the single-unit meal conducted at the lower temperature.

The multiple-unit meal of mealworms was 2.7 times larger in terms of digestible organic matter than the single-unit meal, but the rate of disappearance of food from the stomach remained relatively constant for the several time intervals for each meal. Fish fed the single mealworm digested an average of 3.1 mg/hr, whereas those consuming three mealworms digested an average of 6.9 mg/hr. The rate of gastric digestion is apparently related to the amount of food present. As the size of the meal is increased, the amount of food digested per unit time is increased.

As the amount of digestible organic matter increased by 2.7 times, the actual rate of gastric digestion increased 2.2 times. This is in very close agreement with the findings of Hunt (1960) for multiple-unit meals of minnows fed to gar. Hunt found that as the size of the meal increased by a factor of three, the amount of food digested per unit time approximately doubles.

Digestion of proteins. The rate of protein digestion in the stomach was followed by determining the amount of nitrogen in the food and the amount of nitrogen in the stomach remains for each of several time intervals (Table 3). The amount of nitrogen was converted to protein by multiplying by the factor 6.25. The average multiple-unit meal of mealworms contained 110.6 mg of protein.

TABLE 3. Protein digestion in the stomach of the bluegill sunfish fed a multiple-unit meal of three mealworms. Nitrogen determinations by micro-Kjeldahl analysis. Chitin nitrogen excluded from protein calculations. Values represent an average for five fish at each sacrifice interval.

Sacrifice interval (hrs)	Nitrogen in food fed (mg)	Amt. protein fed (mg)	N stomach remains (mg)	Amt. protein digested (mg)	Rate protein digested (mg/hr)	Protein digested (%)
6	17.2	107.5	12.3	26.9	4.5	25.1
10	18.6	116.2	10.3	51.9	5.2	44.7
14	17.9	111.9	8.0	61.9	4.4	55.3
18	17.4	108.8	6.2	70.0	3.9	64.3
22	17.4	108.8	2.8	90.0	4.1	82.7

During the first six hours 25.1% of the protein intake was digested, absorbed, and/or passed into the intestine. Moreover, 44.7, 55.3, 64.3, and 82.7% protein digestion values were obtained for the 10-, 14-, 18-, and 22-hour sacrifice intervals. The decrease of protein and digestible organic matter from the stomach were closely parallel. A calculation of the amount of protein digested per unit time is relatively constant for each hour that food is present in the stomach. The average rate of protein digestion was 4.5 mg/hr for the five consecutive time intervals.

The food remaining in the stomach was still in a solid or semisolid state, while that in the intestine and pyloric caeca appeared as a yellow, creamy, viscid mass, suggesting the final stages in protein hydrolysis. Only very small amounts of protein were found in the intestine at any time interval, supporting the findings of Gerking (1955), who reported that the efficiency of protein absorption for bluegills is about 97%.

The nitrogen in the stomach upon which the protein values are based undoubtedly did not all come from the meal. The amount of nitrogen present in the digestive enzymes or in the bacterial flora is not known; consequently, the calculated values for protein digestion may be slightly low.

Force-feeding. The difficulty encountered in training large numbers of fish to consume simultaneously a measured meal in the laboratory was responsible for the origin of the force-feeding method. Whether the force-feeding technique of previous workers had an effect upon the rate of digestion in fish was never determined, although the sensitivity of fish to even slight environmental change and handling is well known.

Teleosts, in general, are amazingly sensitive to temperature, light (Breder, 1951), and various other external and internal stimuli. Evidence of this can be observed easily by the excited actions of newly caught fish placed in a holding tank. There is no doubt that this excited condition affects various physiological processes, evidenced by the increased defecation and rapid accumulation of waste upon the bottom of holding tanks. Aquarium transfer of goldfish without other treatment produced a hyperglycemic condition, and blood sugar did not return to normal for a period of four days (Chavin, 1964). These physiological and psychological conditions are manifest in other characteristic responses.

One of the initial effects of the manual manipulation of force-feeding is either immediate or delayed regurgitation. The occurrence of regurgitation upon force-feeding minnows to largemouth bass was reported by Molnár and Tölg (1962). Numerous problems connected with feeding and handling occurred in Plantenberg's (1961) experiments with regurgitation complications. Markus (1932) encountered problems connected with force-feeding but mentioned no regurgitation. Hunt (1960) had to force-feed gar in order to obtain results comparable with the warmouth bass (*Chaenobryttus gulosus*) and largemouth bass, which fed naturally. Hess and Rainwater (1939) reported no regurgitation when trout were force-fed. Bluegills held at temperatures of 17 and 20°C only rarely regurgitated force-fed mealworms in Malcolm's (1960) experiments. Some of the pike (*Esox lucius*) regurgitated force-fed perch before the time of stomach pumping in the digestion experiments of Seaburg and Moyle (1964). This regurgitation was not uncommon in the following force-feeding experiments.

On the supposition that force-feeding has an effect upon the physiological body processes of fish, the following preliminary digestion experiment was designed. Each of five fish of the same relative size and weight was quickly removed from the aquaria, and with a blunt forceps a single mealworm placed in the stomach. A similar group of five control fish was allowed to voluntarily consume a single mealworm. The average live weight of the mealworm larvae consumed was 121.4 mg. All the fish were sacrificed 18 hours after feeding.

The average decrease in stomach contents for the control fish after 18 hours was 63.1% with a range of 48.4-78.3%. The average decrease in stomach contents for the force-fed fish was 82.1% with a range of 52.8-100%. The results suggest that force-feeding may not only increase the rate of digestion but also cause increased variation among individual fish.

In order to explore this problem further, a second force-feeding experiment was designed. A group of 30 fish was pre-conditioned to feed on mealworms before beginning the three-day fasting period. At the end of this period one group of 15 control fish was fed a multiple-unit meal of four to five mealworms per fish with an average organic weight of 169.2 mg. A second group of 15 experimental fish was simultaneously force-fed an average of four to five mealworms per fish with an average organic weight of 173.7 mg (Table 4).

The rate of digestion of force-fed fish was considerably reduced in comparison to the voluntary-feeding fish at the 6- and 10-hour sacrifice intervals (Fig. 2). One explanation for the decrease in digestive rate is the manual manipulation of force feeding. No significant difference was found between the average percentage decrease of stomach contents for the two groups at the 14-hour interval. Nevertheless, it is significant that the coefficient of variation at the 14-hour interval for the force-fed fish was 40.8%, whereas the coefficient for the naturally feeding control fish was 2.8%. On the average the force-fed fish had higher coefficients than those fish that fed voluntarily at all sacrifice intervals.

The average decrease of digestible organic matter in the stomachs of the control fish in order of increased sacrifice time (6, 10, 14, 18, and

TABLE 4. Force feeding and the rate of digestion for the bluegill sunfish. Totals for all fish, 15 individuals in each group.

	No. meal-worms	Av. fish length (mm)	Av. fish weight (g)	Av. live weight of food (mg)	Av. organic weight of food (mg)	Food as % of body weight	
						Live weight	Organic weight
Control fish (voluntary feeding)	4-5	136.7	47.6	445.2	169.2	0.94	0.36
Force-fed fish	4-5	141.1	53.0	457.1	173.7	0.86	0.33

22 hours, respectively) was 17.9, 19.1, 48.5, 83.8, and 85.2%. These values compare favorably with the results of the multiple-unit mealworm experiment described above. The decrease in stomach contents for the force-fed fish in like order was 3.8, 22.8, 51.4, 70.4, and 69.7%. The low value at the 6-hour interval was caused by two fish that accomplished no measurable digestion during this period but which appeared normal in all other respects. The remaining fish in this group managed to digest 20.5 mg of organic matter during the 6-hour period, which was 11.3% of the consumed meal. This was lower than any of the control fish at that interval.

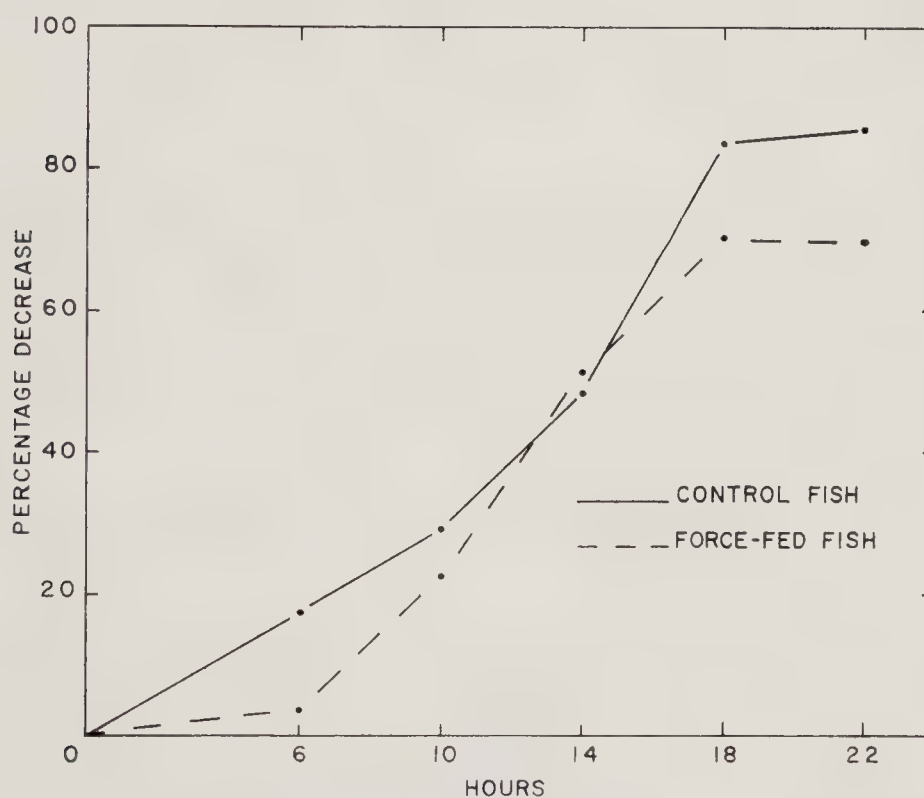


FIG. 2. The average percentage decrease of digestible organic matter from the stomach for both force-fed and voluntarily feeding bluegill sunfish. Each data point represents the mean of 15 individuals.

The high coefficient of variation on the part of the experimental group is attributed to the effect of force-feeding. A combination of factors may be involved. The manual manipulation of force-feeding may cause psychological disturbances, which in turn affect certain physiological body processes. The effects can be exhibited in an increased rate of digestion as found in the preliminary force-feeding experiment or as a decreased rate as in this experiment. In either case the increased variation is obvious, and prediction of the outcome of such experiments cannot be made with certainty.

Age and size of the fish. Karpevitch and Bokoff (1937) reported that age of the animal is an important factor in fish digestion. If rates of digestion vary with differences in age and size of the animal, this factor assumes importance in all future investigations of fish digestion and may have affected the results of earlier studies.

Hunt (1960) reported that large gar when force-fed a single *Gambusia* digested food much more slowly than smaller gar. However, when gar of different sizes were fed a multiple-unit meal of live *Gambusia*, no significant difference was ascertained. Hunt was unable to show a positive relationship between rates of digestion and body weight of the largemouth bass.

Seaburg and Moyle (1964) reported that the overall rates of reduction of stomach contents for Maple Lake, Minnesota, fish were similar for all sizes of bluegills, but in contrast, the rates of reduction for Grove Lake, Minnesota, bluegills differed with the size of the fish and were generally faster than those from Maple Lake. These observations were made on fish caught by seining and held in food-free live boxes. The variable results could have been the result of handling.

The investigation of the relationship of age and size on digestion was not thoroughly done in the present series of experiments, and the results were not statistically reliable due to the small sample size. They are reported, nevertheless, because two experiments provided identical conclusions. Two groups of fish were aged by means of scales. Large fish averaging 118.2 g and 177.6 mm in length were found to be on the average 3.5 years old. Smaller fish averaging 29.8 g and 118.2 mm in length averaged 1.4 years of age. The experimental meal consisted of a single mealworm averaging 171.0 mg of live weight and 65.0 mg of organic weight. All fish were sacrificed exactly 18 hours after consuming the meal.

No significant difference in the rate of digestion was observed when the results of the two groups were compared. The decrease from the stomach after 18 hours was 66.0% for the five large fish and 72.9% for the ten small fish.

The design of a second experiment was identical with that previously described with the exception that multiple-unit meals of mealworms were fed. Each of five bluegills, averaging 197.8 mm in length and 145.4 g in weight, consumed nine mealworms. The meals averaged 1130.2 mg of live weight and 429.5 mg of organic weight. Each of the four small bluegills, averaging 119.6 mm in length and 33 g in weight, consumed three mealworms that averaged in total 384.2 mg of live weight and 146.0 mg of organic weight. Both groups of fish were aged by means of scales. The

large fish averaged 4.5 years of age, whereas the small fish averaged 2 years of age. The decrease of digestible organic matter from the stomach was identical for both groups of fish, and averaged 83.3%.

The difference in age and size of the fish was taken into consideration by adjusting the size of the meal to the body weight. For this reason different amounts of organic matter were digested per unit time. The large fish averaged 19.3 mg/hr, and the small fish averaged 6.8 mg/hr digestion for each of the 18 hours of the experiment.

Starvation. A fasting period is necessary to assure complete removal of all food remains from the alimentary tract before feeding an experimental meal. Unfortunately, most workers have neglected to report the length of the fasting period, and it is, in fact, often not clear if this variable was considered. Darnell and Meierotto (1962) recommend a fasting period of 12 hours but suggest that the time be shortened or lengthened depending upon preliminary stomach analysis. The rates of digestion might be subject to considerable error if the fish were starved either for a considerable period of time or a period insufficient to empty the digestive tract.

The experimental solution to this problem was first attempted by making a preliminary comparison between fasted and non-fasted groups of fish. Both groups of fish were first pre-conditioned to feed upon mealworms in aquaria. After a period of acclimation and successful feeding, a group of seven fish was fasted for seven days, whereas a control group was fed daily up to 24 hours prior to consumption of the experimental meal. The normal 3-day fasting period was eliminated, and the last meal fed to the control fish consisted of softer-bodied mealworm pupae to distinguish them from the larvae that were subsequently fed. The experimental meal consisted of a single mealworm with an average live weight of 152.9 mg and an average digestible organic weight of 57.8 mg. All fish were killed after 18 hours.

The average decrease of digestible organic matter for the six control fish after 18 hours was 79.1%. The average decrease after 18 hours for the fish starved seven days was 70.1%. These preliminary results indicated that starvation decreased the rate of digestion and justified a more thorough investigation of the problem.

Three parallel series of experiments were set up to illustrate quantitatively the degree of the effect of starvation upon the rate of gastric digestion. Groups of 15 fish were starved for 7, 14, and 25 days. Both control and experimental fish were pre-conditioned to feed upon mealworms prior to initiation of the fasting period. The control fish were fed twice daily on oligochaetes, which were easily distinguished in the stomachs from the mealworms. The last meal was consumed 24 hours prior to being fed the experimental meal. The last two meals for the control fish were reduced in amount to eliminate errors in the stomach weight analysis. In each experiment three fish from each group were sacrificed at each of the five time intervals previously mentioned.

All the bluegills consumed a multiple-unit meal averaging 181.3 mg of digestible organic weight and nearly 0.5 g of live weight. The size of each fish was considered in determination of the size of the meal, and only whole organisms were fed. The average length for all fish in the

three parallel series was 128.0 mm, and they weighed an average of 36.6 g.

The rate of digestion of fish starved seven days was decreased by a significant amount at every sacrifice interval in comparison to the values for the control groups. In fact, as the length of the starvation period increased, the rate of gastric digestion decreased (Fig. 3). This significant decrease in gastric digestion after only seven days of starvation agrees with the results of the preliminary starvation experiment. A greater decrease in digestive efficiency occurred between 7 and 14 days than between 14 and 25 days. Perhaps more pronounced physiological effects occur in the initial stages of starvation, which gradually become less pronounced with increased time.

A comparison of the averages for the three parallel series of fish killed after 22 hours shows the striking differences between the control and experimental fish (Table 5). The decrease of digestible organic matter from the stomach for the control fish was 95.6, 89.5, and 100% for the 7-, 14-, and 25-day experiments. In contrast, the values for fish after being fasted for 7, 14, and 25 days was 75.7, 53.9, and 48.9%, respectively. As in the previously described mealworm experiments, the amount of food digested per unit time remains relatively constant over the course of the experiment and is directly related to the amount of organic matter consumed. The experimental group sacrificed after 22 hours in the 14-day experiment, for example, was fed an average of

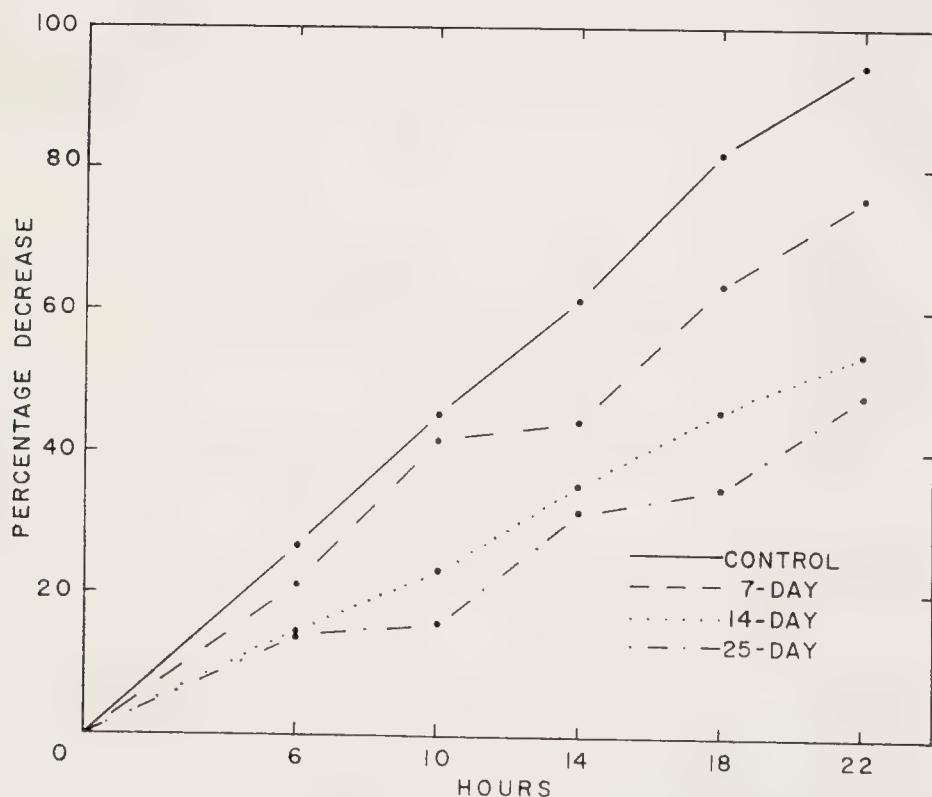


FIG. 3. The average percentage decrease of digestible organic matter from the stomach of bluegill sunfish after 7-, 14-, and 25-day starvation periods. The meal consisted of multiple-unit meals of mealworms. The three control groups have been averaged.

175.7 mg of organic matter. It digested 94.6 mg, and the stomach contents decreased at the rate of 4.3 mg/hr during each of the 22 hours. In comparison, the control group was fed an average of 181.3 mg of organic matter. The fish digested 161.7 mg, and the stomach contents decreased at an average rate of 7.3 mg/hr for each of the 22 hours.

Comparing the average difference in percentage decrease of digestible organic matter at the 22-hour interval shows that the control group digested food 1.26 times faster than did fish starved for seven days. Moreover, those fish starved for 14 and 25 days that were sacrificed after 22-hours on the average digested 1.66 and 2.04 times more slowly than did the control fish. The variation in the average mg/hr digested values for the experimental and control groups is partly due to differences in amounts of organic weight fed.

Careful observation of the digestive tracts of the starved fish showed striking morphological changes in comparison to those of the control fish. These changes were especially prominent in the pyloric caeca, which were noticeably atrophied. The caeca were definitely shrunken after seven days, and the condition became progressively advanced with time. In view of these observations it appears that the caecal condition is correlated with the digestive function and has probably resulted in a decrease in the amount of digestive enzymes secreted. The pyloric caeca have a dual function in fish, being a site of enzyme production (Lawrence, 1950) and an active area of absorption (Greene, 1913; Dawes, 1930). These observations furnish evidence that starvation interferes with the normal functioning of this organ, which in turn directly affects the rate of digestion. The function of the pyloric caeca is not restored to normal over the 22-hour interval of the experiment, since the fish in all three series digested at about the same rate during the last few hours as they did during the initial period.

TABLE 5. Starvation and the rate of digestion for the bluegill sunfish using mealworms as food. The data represent averages for fish sacrificed at 22 hours.

Number days starved		Number fish in experiment		Fish weight (g)	
Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
0	7	15	14	38.0	49.0
0	14	15	15	36.6	34.6
0	25	15	15	36.0	33.3

Amt. organic weight fed (mg)		Amt. digested (mg)		Rate of digestion (mg/hr)		Decrease of digestible organic matter	
Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
173.3	109.1	165.6	80.2	7.5	3.6	95.6	73.5
181.3	175.7	161.7	94.6	7.3	4.3	89.5	53.9
174.0	169.7	174.0	82.9	7.9	3.8	100.0	48.9

DIGESTION OF NATURAL FOOD ORGANISMS

A series of six analogous experiments was conducted for the specific purposes of establishing the relative rates of digestive disintegration, movement, and absorption of different natural food organisms. The bluegills were pre-conditioned to feed upon the natural food item for each experiment prior to a 3-day fasting period. The sample size ranged from 16 to 21 fish in each experiment. The average temperature at which the experiments were conducted was 20.8°C. That recorded for the dragonfly naiad was somewhat lower, 17.6°C (Table 6).

The food organisms were chironomids, crayfish, darters, dragonfly naiads, mayfly naiads, and oligochaetes. These organisms were chosen for two reasons: (1) it is generally believed that soft-bodied organisms, such as oligochaetes and dipterous larvae, might be digested and passed through the alimentary tract much more rapidly than heavily chitinized forms, such as crayfish and dragonfly naiads, and (2) these food organisms have been found in bluegill stomachs.

Multiple-unit meals were fed depending upon the size of the food organism. Forty small chironomids were fed, compared with 3-8 of the larger food organisms. The meals were of the same relative live weight with the exception of the chironomid and dragonfly naiad experiments; the chironomid meal contained the largest average live weight of 858.9 mg, and the dragonfly naiad meal contained the smallest, 205.8 mg.

All the food organisms were voluntarily consumed with the exception of the darters. Unsuccessful attempts at pre-conditioned feeding occasioned the necessity of force-feeding. The feeding technique involved quickly removing the fish from the aquarium, and as rapidly as possible placing the darters into the esophagus with a blunt forceps. Each fish was fed an average of five to eight darters.

In general, all the natural food organisms were digested at approximately the same rate regardless of the chemical composition of the body (Fig. 4). The only major difference between the six experiments was the digestion rate recorded for dragonfly naiads at the 14-, 18-, and 22-hour sacrifice intervals. These slower rates can be explained on the basis of the 3.2°C temperature difference, mentioned above.

TABLE 6. A comparison of the experimental data on digestion of natural food organisms by the bluegill sunfish.

	Number of fish in exper- iment	Fish length (mm)	Fish weight (g)	Number food organ- isms fed	Live weight fed (mg)	Organic weight fed (mg)	Av. temp. (°C)
Chironomids	21	140.8	64.4	40	858.9	90.2	21.5
Crayfish	21	140.8	62.3	3-4	630.8	96.5	22.0
Darters	16	149.5	64.1	5-8	594.6	112.4	20.8
Dragonfly naiads	18	120.2	33.7	3	205.8	37.5	18.1
Mayfly naiads	22	125.5	34.4	3	615.2	110.1	21.0
Oligochaetes	18	124.6	40.4	3-6	553.3	81.9	21.4

If the percentage chitin of a food organism has a major inhibiting effect upon digestion, one would expect this effect to be demonstrated to some degree for each of the chitinized food organisms. In contrast, oligochaetes and darters might be expected to be digested more quickly than the chitinized forms. This is clearly not the case. All food organisms show close relationships in time required for digestion. Crayfish had the highest percentage decrease values at each of the sacrifice intervals. The meal of chironomids was the largest in terms of live weight (858.9 mg) but below average organic weight (90.2 mg), and digestion was completed slightly in excess of 18 hours. The rate of digestion of oligochaetes falls between the curves for the chitinized organisms. The percentage decrease for dragonfly naiads at the 6- and 10-hour intervals is not essentially different from any of the other food organisms and shows a very close relationship to the curves for chironomids and mayfly naiads. In other words, these organisms were all digested at about the same rate for the first 10 hours, in spite of the fact that considerable differences exist in the percentage chitin of the live weight of the organisms (Table 1). As previously noted, chironomids have the smallest, mayfly naiads an intermediate, and dragonfly naiads the largest percentage of chitin of the organisms analyzed.

Temperature records taken during the course of the dragonfly experiment show that the temperature dropped from 18.5°C at feeding to 17.1°C at the 14-, 18-, and 22-hour sacrifice intervals. The major decrease in the rate of gastric digestion of dragonfly naiads is attributed to this uncontrolled temperature drop during the course of the experiment.

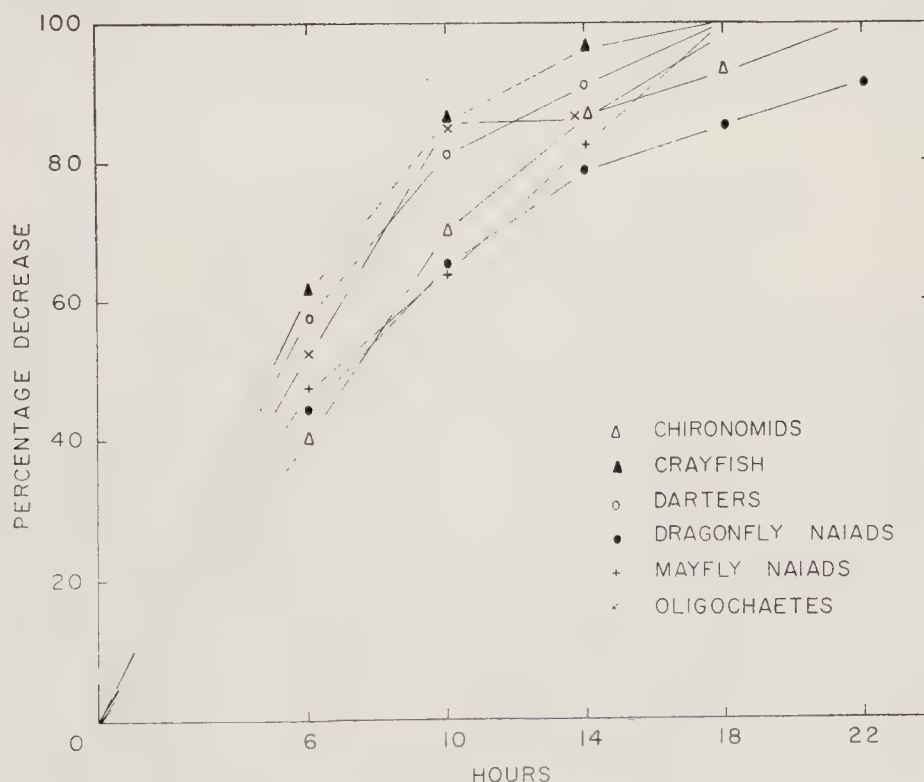


FIG. 4. The average percentage decrease of digestible organic matter from the stomach of bluegill sunfish after voluntarily consuming meals of natural food organisms. Only the darters had to be force fed.

Excluding the dragonfly naiad experiment, the average decrease of digestible organic matter from the stomach for all the food organisms combined at the 6-hour sacrifice was 50.5%. Likewise, the combined decreases for the 10-, 14-, 18-, and 22-hour intervals were 77.4, 88.8, 98, and 100%, respectively.

The percentage decrease of food from the stomach at the 18-hour sacrifice interval gives the best clue to the time when the stomach becomes completely empty for the bluegill after consuming multiple-unit meals of natural food organisms. The meals of mayfly naiads, crayfish, and darters all reached 100% digestion by the 18-hour sacrifice. The meals of chironomids and oligochaetes reached 92.9 and 97.8% digestion, respectively. The meal of three dragonfly naiads, because of the temperature drop, reached only 85% digestion after 18 hours. This information indicates that about 17 to 19 hours is required for the stomach of the bluegill to empty after consuming meals of natural food organisms at water temperatures of 21°C. This compares closely with the rate of digestion for the largemouth bass. Hunt (1960) reported that the time required for the largemouth bass stomach to empty after being fed five *Gambusia* ranged from 16 to 19 hours.

The fact that mealworms require a longer gastric digestive period (22-26 hours) than natural food organisms (18 hours) is a question that has not been resolved during the present research. The discrepancy may be partially explained by the fact that no direct comparison was ever made between the rates of digestion of mealworms and natural food organisms at the same time and with the same stock of fish. The mealworm tests were performed on specimens from southern Indiana, and the tests on natural food organisms were made on fish from northern Indiana. A direct comparison under carefully controlled conditions is required to clarify this problem.

*Digestion of a voluntarily consumed mixed
meal of natural food organisms*

This experiment is an initial attempt to examine quantitatively the rate of digestion of a mixed meal of natural food organisms. The experiment was organized and conducted in a manner similar to the previously described experiments under identical laboratory conditions. Bluegills that averaged 139.8 mm in length and 56.7 g in weight were pre-conditioned to feed on chironomids, oligochaetes, and dragonfly naiads. After a short period of acclimation and a 3-day fasting period, each fish was allowed to consume voluntarily 25 chironomids, one dragonfly naiad, and one oligochaete. The meals averaged 821.6 mg of live weight and 91.0 mg of organic weight. The average temperature during the critical period of digestion, *i.e.*, from the time the meal was fed to the time of the last sacrifice, was 24.1°C.

The average percentage decrease of digestible organic matter from the stomach for the meal shows a close relationship to the average totals for the previously described natural food experiments. The average percentage decrease for the mixed meal was 63.8, 85.5, 90.0, 85.5, and 93.5 for the 6-, 10-, 14-, 18-, and 22-hour sacrifice intervals, respectively

(Fig. 5). As before digestion was most rapid during the first 6 to 10 hours, with only about 15% of the original experimental meal remaining after the 10-hour sacrifice. Similarly, only 10% of the mixed meal remained in the stomach after 14 hours of digestion. This compares very closely with the combined total average for the six natural food experiments of 11.2%. Moreover, these results agree with that from a field study by Seaburg and Moyle (1964) for 125 to 150 mm bluegills that consumed all types of food during the entire summer season. Interpolated time values for 100% volume reduction to occur was 20 hours.

No differences were observed at autopsy in the relative rate of digestion of the different food items. The digestible organic matter of chironomids, oligochaetes, and dragonfly naiads all disappeared from the stomach at the same relative speed. The rate of passage of the food items through the stomach was the same, since all food material passes from the stomach to the intestine as a soluble chyme. The smaller chironomids were not digested any more rapidly than the larger oligochaetes. There was, however, a characteristic lag in the passing of chitin from the stomach as a result of feeding the highly chitinized dragonfly naiads.

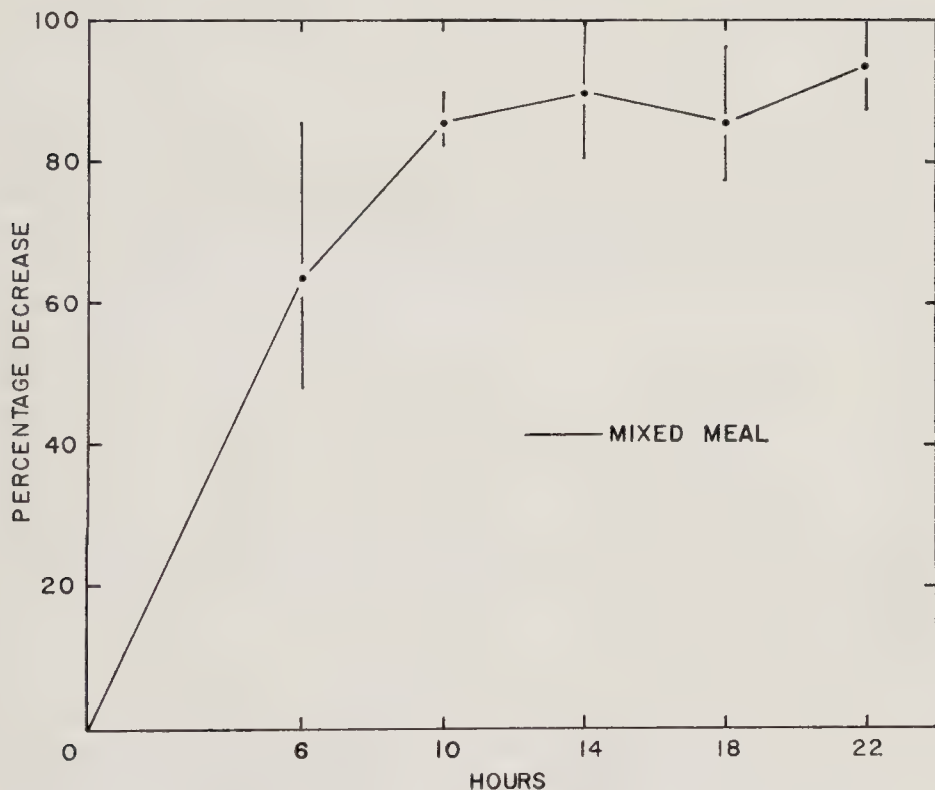


FIG. 5. The average percentage decrease of digestible organic matter from the stomachs of bluegill sunfish after voluntarily consuming a mixed meal of natural food organisms. The mixed meal was composed of 25 chironomids, 1 dragonfly naiad, and 1 oligochaete. The vertical lines represent the range of the percentage decrease for the four fish at each sacrifice interval.

ABSORPTION

The rate of digestion in fish is known to be much slower than in mammals, and the processes of absorption are bound to be influenced by the rate at which food passes through the alimentary tract. The control mechanism governing the rate of peristalsis has not been determined, yet it is clear that the rate of digestion, absorption, and peristalsis is affected by a number of factors including temperature, amount of food consumed, nature of the food, condition of the animal, stress, etc.

In the present experiments an attempt was made to measure the rate of absorption and its relation to digestion up to the time of the first defecation. In each experiment the dry weight of the intestinal remains was determined separately from the dry weight of stomach remains, and these values were used to calculate rates of absorption. The average amount of absorption for each time interval was computed by subtracting the sum of the stomach and intestine remains from the amount of organic matter consumed. The appropriate correction for indigestible chitin was made.

The reported absorption data are accurate only up to the time of the first defecation, since after this point it is not known how much food had been absorbed and how much had been removed as feces. The time of the first defecation for the bluegill has been observed to occur about 10-14 hours after ingestion of a meal. This observation was made on fish fed an experimental meal after a 3-day fasting period. The time of the first defecation after feeding under natural conditions may be considerably sooner or later depending upon the species considered, food consumed, and environmental conditions. Dobreff (1927) noted defecation first occurring in dogfish five days after consuming a meal.

There is a high correlation between the rate of absorption and the rate of digestion, as would be expected. The amount of food digested and the amount of food absorbed in mg for the multiple-unit mealworm and mayfly-naïad experiments (Table 7) compares favorably with the results for other organisms. The amount of absorption follows the amount of digestion very closely, and both increase with time. The amount of absorption at each time interval was slightly less than the amount of food digested. This slight difference is attributed to the amount of food that is present in the pyloric caeca and which was impossible to remove by the methods used.

The absorption values represent the total for the stomach and intestine. The actual amount of absorption taking place in the stomach is unknown for the bluegill, but there is positive evidence for gastric absorption in fish (Van Slyke and White, 1911; Greene, 1913; Dawes, 1930; Mathews and Parker, 1950). These experiments indicate that gastric absorption also takes place in the bluegill sunfish. Considerable hydrolysis was observed to occur after six hours of digestion, yet it appeared that the passage of food into the intestine had hardly begun as evidenced by the small amounts found. Consequently, a rather large amount of food was left unaccounted for, implying either gastric absorption, or immediate intestinal absorption.

TABLE 7. Rate of digestion and absorption for the bluegill sunfish. These data represent an average of the fish at each sacrifice interval for the multiple-unit mealworm and mayfly naiad experiments.

Time interval (hrs)	Total digested (mg)	Total absorbed (mg)	Rate of digestion (mg/hr)	Rate of absorption (mg/hr)
Mealworm				
6	34.8	26.1	5.8	4.4
10	71.6	62.2	7.2	6.2
14	99.3	90.4	7.1	6.5
18	128.3	120.1	7.2	6.7
22	153.3	144.6	7.0	6.6
Mayfly Naiad				
6	42.1	34.0	7.0	5.7
10	69.7	61.2	7.0	6.1
14	93.6	84.7	6.7	6.1
18	*110.7	110.7	6.2	6.2
20	—	—	—	—

* Represents 100% digestion

The shrinkage of the alimentary canal caused by the prolonged period of starvation reduced the area of absorption, which should decrease the efficiency of the absorptive processes. A comparison of the amount of food digested with that absorbed for starved fish indicated that the absorption processes remain adequate to meet the demands of the digestive processes. No accumulation of gastric or intestinal chyme was observed or detected by dry weight analysis.

DISCUSSION

Factors influencing rate of digestion

In addition to the variables that affect the rate of digestion discussed earlier, special consideration needs to be given the problems of (1) sensitivity of fish to handling, (2) size of the meal, (3) meal succession, (4) differential rates of digestion of natural food organisms, and (5) starvation.

Handling and fish sensitivity. In two of the three experiments reported here on force-feeding, an increase in variation was observed in the results when compared with the results of fish that fed voluntarily. The increased variation decreases reliability in the outcome of such experiments. On some occasions, force-feeding resulted in regurgitation, and on other occasions intact mealworms were found in the intestine. The third experiment concerning force-fed darters resulted in much less variation than the usual experiment. This conflict cannot be resolved without further experimentation.

The increased variability is not surprising in light of recent discoveries relating a high degree of fish sensitivity to handling. LeLoup-

Hatey (1958) and Chavin and Kovacevic (1961) demonstrated that handling produced histochemical depletion of goldfish adreno-cortical cells. The suggestion was made that most workers are not aware of the stress to which fish are subjected when improperly handled. The present investigation supports these studies. The data clearly show a relationship between handling and digestive physiology.

Digestion of large and small meals. Based on the gastric acidity and pH work of Vonk (1938, 1939), Barrington (1957) suggested that a smaller meal may be expected to be digested more readily than a larger one. This is clearly not the case for the range in sizes of meals fed to the bluegill sunfish. These experiments, and those of Hunt (1960), conclusively show that as the size of the meal is increased the amount digested per unit time also increases. The digestive system probably increases enzyme production with increased food volume in the stomach. Nevertheless, the fact that the stomach empties at about the same rate for both single- and multiple-unit meals of mealworms raises interesting problems about gastric motility. If the rate of peristalsis under normal conditions is relatively constant, then the amount of food consumed may be limited. Additional knowledge about the secretory and motor control of the alimentary canal of fish may solve many problems with regard to rate of feeding, digestion, absorption, and utilization.

Barrington (1957) also suggested that small prey might be digested more quickly than large prey. This was not the case for the digestion of natural food organisms. Meals of small prey, *e.g.*, chironomids, were digested at about the same rate as meals of large prey, *e.g.*, oligochaetes. This was in spite of the fact that individually each oligochaete was over nine times larger than each chironomid.

Meal succession. Dawes (1930) and Karzinkin (1935) reported that the rate of food passage is related to the amount of food recently consumed, *i.e.*, the sooner the second meal is consumed the more quickly the first meal leaves the stomach and passes down the alimentary tract. This infers that whole or partially digested food organisms are apt to be found in the intestine or even defecated when successive meals are consumed. This is clearly not true in the present case.

Prior to each digestion experiment attempts were made to determine the number of food organisms that could be fed without rejection or undue forcing. Bluegills were fed as much as they would consume and then offered additional food at intervals. Although the stomach is highly elastic, there is an upper limit to the maximum ration. Once the stomachs were full, additional food items were rejected. As the stomachs emptied (with time) the fish were able to eat additional food. The size of the specific food organisms consumed was related to the available space in the stomach. This may mean that small organisms such as chironomids could be consumed more quickly after a full meal than an organism the size of *Hexagenia*. At no time were undigested or whole food items defecated.

Similar observations were made by Moore (1941), who measured the amount of food eaten by the green sunfish, *Lepomis cyanellus*, the perch, *Perca flavescens*, and the bluegill, and found even with an unlimited food supply that only a limited amount was eaten daily. Brown

(1951) observed that *Salmo trutta*, the brown trout, has a definite appetite and will not eat more food even when plenty is available.

Differential rates of digestion of natural food organisms. Knowledge about rates of digestion of different types of meals by the bluegill offers a fuller understanding of the ecology of the species in respect to feeding, maintenance, and growth. Single-species and mixed-meal experiments did not differ markedly with regard either to rate of passage of food through the stomach or rate of digestive disappearance from the stomach. Upon inspection of the stomach and intestine during autopsy, no differences were observed in the relative digestibility of the different food organisms. Organic matter was digested and disappeared first. Chitin lingered in the stomach for a longer period of time, until broken into small pieces. In nature, additional consumption of food during continuous feeding may aid in the removal of chitin from the stomach more quickly. Gerking (1962) suggests that the indigestible plant material may be used for "roughage," aiding digestion by scouring out the head capsules and other chitinous debris that may accumulate in the stomach. This "roughage" may have other unknown functions in the bluegill. Jancarik (1964) pointed out that the natural food of carp, *i.e.*, *Daphnia*, tubificids, earthworms, and mosquito larvae, helps regulate certain aspects of their digestion. He found that complete digestion of starch in carp can be ensured only by a sufficient supply of animal food. Plant material in the bluegill diet may have a similar function.

Starvation and the winter period. The starvation phenomenon of fish assumes considerable ecological significance with regard to the winter period in temperate lakes. Bluegill sunfish are known to feed during the winter as evidenced by food habit studies and catches of ice fishermen. Moffett and Hunt (1943) analyzed the stomach contents of 1135 bluegills in Cedar Lake, Michigan, and found that during the winter period from 18 January to 18 March an average of only 0.036 ml volume of food per stomach was present. This is in contrast to the quantity found at other seasons. Parks (1949) found an average stomach volume of 0.34 ml for bluegills averaging 138 mm fork length caught by anglers in August from Winona Lake, Indiana. This is a 10-fold increase over the winter season. The small volumes of food in the stomachs of bluegills in the winter are not surprising when factors such as temperature and starvation are considered. On the basis of digestion results obtained at 5°C and 25°C, Molnár and Tölg (1962) concluded that the rate of emptying of the stomach of largemouth bass is 5 to 6 times slower in winter than in summer. Hess and Rainwater (1939) reported that a temperature drop to 1.9°C at the end of their fish digestion experiment brought digestive activity almost to a standstill, implying a similar difference in rate of feeding. Bluegills held at 4°C in the laboratory required more than seven days for digestion of a single mealworm (Malcolm, 1960). These temperature studies reflect the seasonal changes in the intensity of digestion. Since the bluegill digestion rate for natural food organisms was established at about 18 hours, the works of Molnár and Tölg and of Malcolm suggest a winter rate of 6 to 7 times longer or about 108 to 126 hours.

The shrinkage of the pyloric caeca discovered in these experiments undoubtedly occurs in nature during fasting periods and may occur each winter. This suggests that the rate of digestion in winter not only decreases as a result of temperature, but partial or complete starvation with the associated morphological changes in the pyloric caeca also may be a factor. A decrease in enzyme production could result from the decreased surface area of the pyloric caeca. It may be a physiological necessity for fish to consume some food during the winter period to insure maintenance of a normal physiological level of gastric digestion.

The decrease in digestive efficiency with an increased length of the fasting period might have been predicted on the basis of earlier work. Barrington (1957), referring to Dobreff's (1927) work on the measurement of gastric acidity of elasmobranchs, stated, (p. 126) "... this [HCL] continued to be produced for as long as 112 days, and, in fact, until the death of the animal. During this period the acidity tended to fall, but the acid was still being secreted in detectable quantity even at the end. If a fasting animal were allowed to feed, the acidity of the gastric contents rose, but the rise was a gradual one and the maximum acidity was not attained until up to four days after the ingestion of the food . . ." The time of recovery from the effects of starvation was not studied in the current bluegill experiments nor has it been for other teleosts. Partial starvation periods may occur in nature both in winter and summer. The large number of empty stomachs found in food-habit studies may imply undernourishment. At the culmination of the winter period the bluegills may be able to make only gradual digestive adjustments to increased temperatures and food. It would appear from the work of Dobreff (1927) that the recovery period may be four days.

The daily ration

The daily ration can assume considerable ecological significance with respect to the estimation of the food turnover. The amount of digestible food consumed during the growing season largely determines the growth and production rates of fish populations. An accurate measure of the rate of digestion might be one way by which the food turnover could be estimated. The maximum and minimum daily rations would be the most useful values to obtain. Both could be determined by laboratory studies utilizing the methods outlined in this paper, and field observations could be compared with them. The maximum meal could be established by feeding to satiation, and the maximum rate of digestion would be based on such a meal. The minimum daily ration would be the amount that would just balance weight loss.

"Daily meal" and "daily ration" both refer to the amount of food consumed. The term "daily meal" was first used by Surber (1930). Soon after, Bajkov (1935) proposed a field method for estimating the total, daily, seasonal, and annual meal of fishes. This method involves establishing the rate of digestion in the field for the species concerned. The rate of digestion was defined by Bajkov as the number of hours necessary for passing all the food (present under natural conditions at that time)

from the stomach into the intestine. Once the rate of digestion is established, the size of the daily meal can be calculated by the formula $D=A \cdot 24/n$, where D =the daily consumption during the time of experiment, A =the average amount of food in the stomach at the time of the experiment, and n =the number of hours necessary for passing all the food from the stomach into the intestine.

Establishment of the size of the daily meal enables the "daily ration" to be computed. Ricker (1946) defined the daily ration as the size of the daily meal expressed as a percentage of the body weight. This concept of the daily ration was used to judge the efficiencies of food in hatcheries by Deuel *et al.* (1942).

Once the laboratory data on maximum and minimum rations become available, field information could be gathered for comparative purposes by techniques similar to those described and utilized by Bajkov (1935), Darnell and Meierotto (1962), and Seaburg and Moyle (1964). Additional data on the diurnal feeding habits of the species would have to be known for accurate computation of what could be called the "actual daily ration" in nature. Such comparisons will lead to ecological predictions concerning both fish and fish food populations in relation to growth and production in aquatic ecosystems.

The calculation of the daily ration can be illustrated by using the experimental data from the mixed-meal and natural-food experiments. To calculate the daily meal, the amount of food digested during the first six hours was multiplied by the number of feeding periods in a day. This is based on the assumption that bluegills are continuous visual feeders and that a maximum of three 6-hour feeding periods can be utilized per day.

The average amount of food consumed with the mixed meal experiment was 822 mg of live weight. After six hours of digestion the stomach contents decreased by 63.8%, or 524 mg. The estimated daily meal would be $524 \text{ mg} \times 3 = 1.57 \text{ g}$. This value represents the amount of food that could be consumed and digested over the 18-hour period based on the size of the experimental meal. Dividing the size of the daily meal by the average body weight of the fish of 56.7 g gives a daily ration of 2.8%.

Making the same assumptions as above, and assuming about 50% digestion of chironomids in six hours, a daily ration of 2.0% was computed. A similar value was obtained when the combined totals for the six natural-food experiments were used.

These daily ration values differ from one another for several reasons. The major difference is accounted for by variation in the amount of live weight of the different meals, thereby influencing the calculation of the daily meal. Computation of the daily ration on the basis of different average body weights of the fish for the several experiments caused additional discrepancy.

None of the above estimated daily rations can be considered maximum values, since the fish were not fed to satiation. Gerking (1955) fed bluegills of 26 g average weight close to the maximum daily ration of mealworms. This amounted to 3.59% of the body weight per day over a period of 30 days. A maximum daily ration of natural food organisms

would be expected to be higher than this, since they have a higher water content than mealworms. Seaburg and Moyle (1964), using a method similar to Bajkov (1935), calculated daily rations of 1 to 2% for bluegills and other centrarchids in Grove and Maple Lakes, Minnesota. These values appear low in comparison with those estimated for the mixed meal and with the value obtained in Gerking's feeding experiments. The comparison corroborates Seaburg and Moyle's opinions that the fish were living below a dietary maximum.

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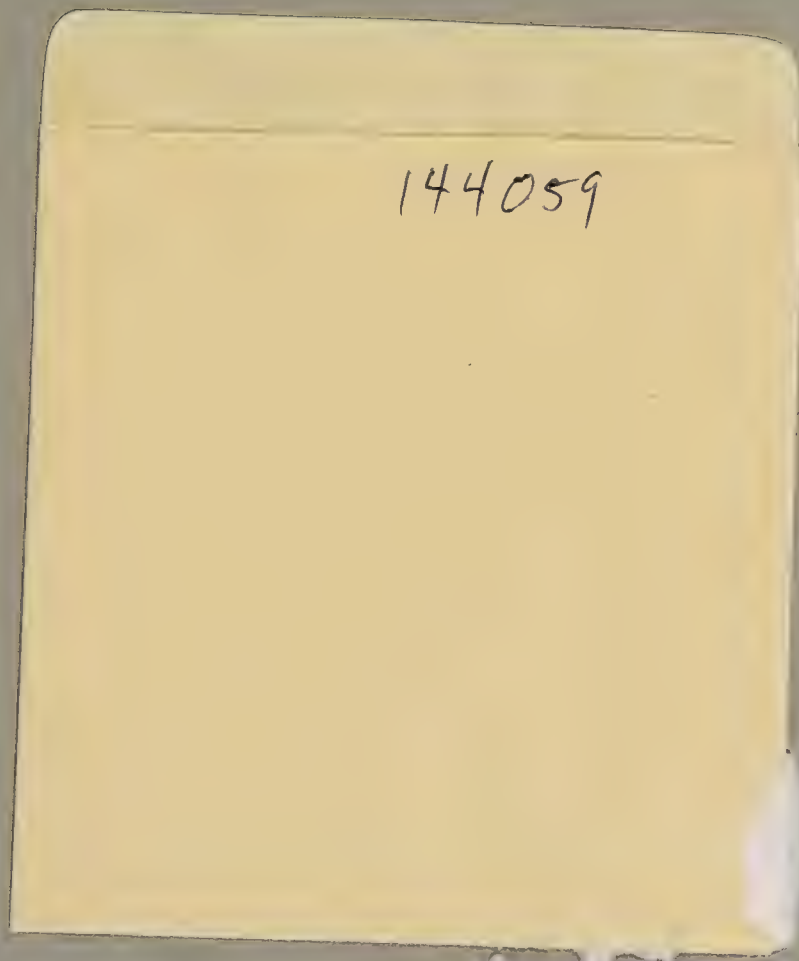
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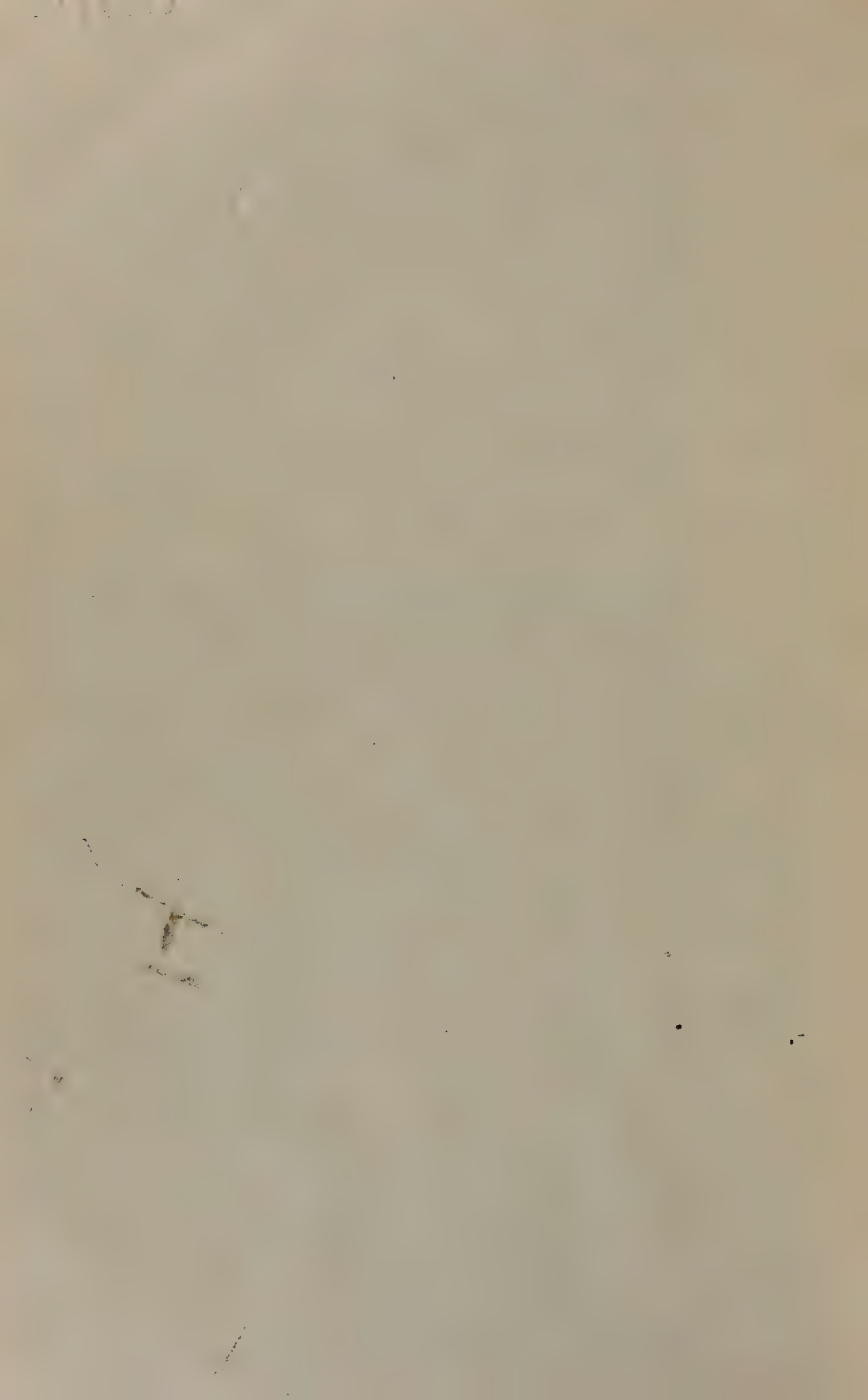


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